a preliminary investigation using an artificial ecosystem SIVA-III [19] of our previous design and obtained results that would suggest the robustness of the above hypothesis [10, 22]. Thereafter, we constructed a more sophisticated model for a more detailed investigation making use of an AChem ecosystem SIVA-T05 [24]. The essential questions we sought to answer were as follows: Would an individual mortal organism, overwhelmed by immortal organisms, become extinct, or could such an individual survive and produce offspring? If it survived and produced offspring, what kind of power relationships would be established between such mortal organisms and the immortal ones?

Our findings suggest that a mortal mutant individual, born in an ecosystem where only indigenous immortal organisms exist, cannot repeat self-reproduction steadily and thus becomes extinct in most cases. Nonetheless, some mortal mutant individuals in our study did manage to survive at an extremely small but not negligible rate. Moreover, the offspring of these mortal mutant individuals that survived extended their habitation area, surpassed immortal organisms, and prospered without exception. This article discusses the above findings in detail.

2 Methods

2.1 Design of the SIVA Virtual Environment

In the present study, we again used SIVA-T05 as an evolution simulator. Its construction and functions are the same as those utilized in an earlier report [24]. In short, the virtual environment of SIVA-T05 consists of a lattice of spatial blocks on a two-dimensional plane (Figure 2). Environmental conditions regarding temperature, energy, and four kinds of virtual inorganic biomaterials were independently defined for each spatial block. The initial configuration was identical to that in the previous report [24].

2.2 Structure and Behavior of Artificial Life in SIVA

2.2.1 Structure of a Virtual-Life Individual

As in the earlier report [24], we designed a virtual-life individual (VLI) based on Oohashi's self-reproductive, self-decomposable (SRSD) automaton model (Figure 3), which took von Neumann's self-reproductive automaton model [37] as its prototype. Oohashi's automaton G is described as $G = D + FZ + I_{D+FZ}$, where $D = A + B + C$. Here, automaton A produces automata according to instructions on data tape I (that is, a virtual genome). Automaton B reads and replicates data tape I. Automaton C sets the copy of data tape I replicated by automaton B into new automata produced by automaton A and separates these as automaton D. Automaton FZ, which is a modular subsystem plugged into automaton D, decomposes the whole automaton G into components suitable for re-utilization when automaton G encounters serious environmental conditions in which it is unable to live or has reached the end of its life span. Data tape I_{D+FZ} carries an instruction describing automaton D + FZ. Thus, automaton G, which corresponds to $D + FZ + I_{D+FZ}$, can reproduce an identical automaton G as well as decompose itself.

We designed artificial life based on AChem so as to actualize the above-mentioned logical actions and, as faithfully as possible, to reflect the principles of terrestrial life and its subsequent reproduction (Figure 4). That is, a VLI is constructed from four classes of virtual biomolecules: virtual inorganic biomaterials (VI), virtual organic biomaterials (VO), virtual biological monomers

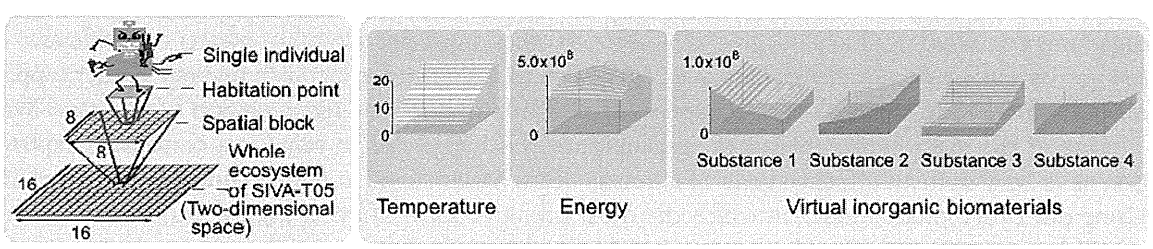

Figure 2. The finite and heterogeneous environmental conditions of the virtual ecosystem SIVA-T05 [24].

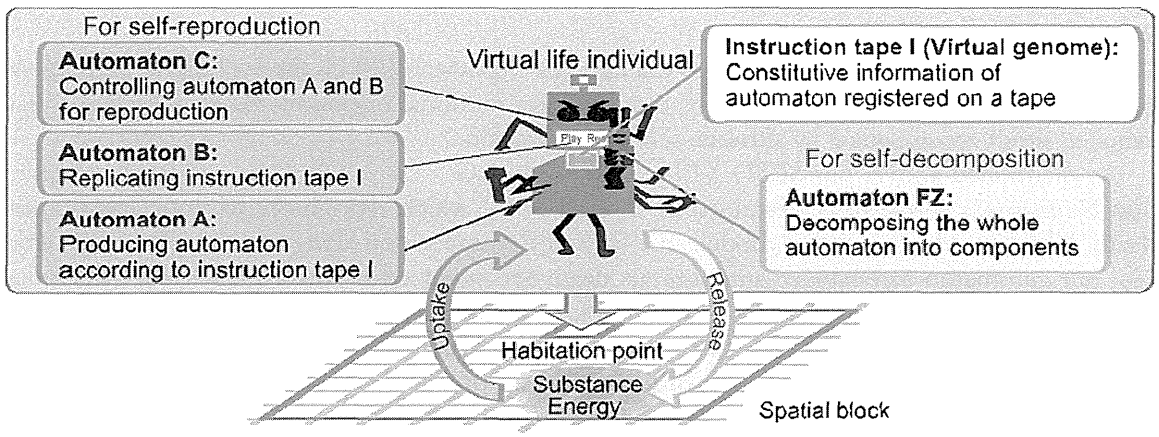

Figure 3. The relationship between life activities of virtual-life individuals (VLIs) and the environment in SIVA-T05 [24].

(VM), and virtual biological polymers (VP). There are four kinds of VI; moreover, any molecules in the latter three classes consist of combinations of the four kinds of VI. A virtual genome in the VP class consists of virtual nucleotides belonging to the VM class. The virtual protein in the VP class is produced according to a sequence of virtual nucleotides that determines the primary sequence of virtual amino acids belonging to the VM class. We developed a SIVA language that actualizes virtual-life activities by recognizing the sequence of the virtual amino acids contained in the virtual protein as coded program sentences and executing therewith. According to given conditions, this SIVA language reproduces, divides, and decomposes a VLI.

2.2.2 Behavior of Virtual-Life Individuals

A VLI executes its life activities by consuming materials and energy from the virtual environment [24]. Activities of each VLI are so designed as to depend on the amount of material and energy available as well as the temperature in the inhabited spatial block. Namely, optimum environmental conditions are defined for each VLI a priori. Activities of a VLI decrease when environmental conditions of the habitation point move away from VLI optimum points. A VLI cannot express its life activities when environmental conditions markedly deviate from the optimum, and, in the case of a mortal organism, it decomposes itself just as it does when it has lived out its life span. Materials and energy released by the decomposition of a VLI are restored to the environment and become utilizable by other individuals within the same space as that occupied by the VLI.

When VLI reproduce, point mutation can occur at a predefined probability during replication of the virtual genome. Mutations may alter the optimum environmental conditions of a VLI. This enables the VLI to live in an environment where it originally could not live. That is to say, evolutionary adaptation to the environment can occur.

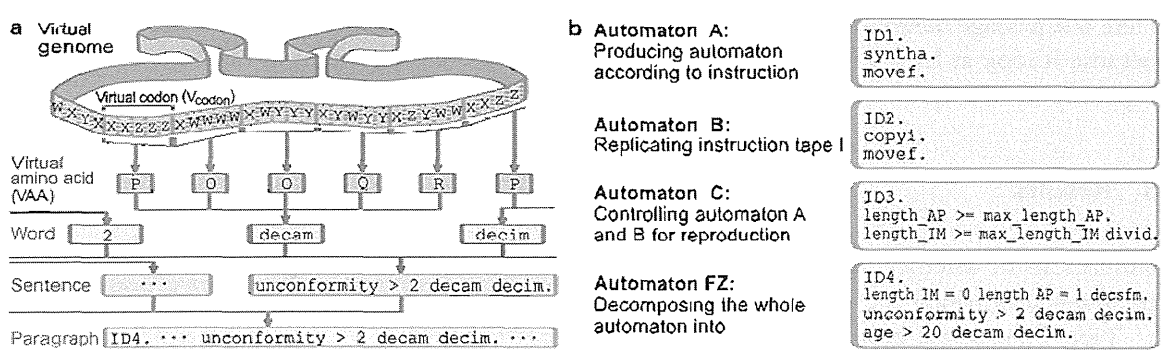

Figure 4. The concept and examples of SIVA language statements that describe the self-reproductive, self-decomposable VLI in SIVA-T05 [24]. (a) Synthesis of virtual protein based on a virtual genome. (b) SIVA language statements describing life activities of automata constituting a SRSD VLI.

2.3 Experimental Setting

We constructed an experimental model to examine our hypothesis [18] on the evolutionary emergence of an altruistic gene using ALife: namely, that primitive immortal organisms through evolution have acquired death accompanied by altruistic self-decomposition.

As mentioned above, our SRSD VLI is described as $G = D + FZ + I_{D+FZ}$. Its prototype is von Neumann's automaton, described as $E = D + I_D$, which reproduces without autonomous death. With the added function module FZ for self-decomposition along with its genetic program I_{FZ}, our automaton can achieve autonomous death accompanied by altruistic self-decomposition. Consequently, mortal organisms are more complex than immortal organisms in terms of structure and function. Von Neumann stated as a matter of principle that "when an automaton performs certain operations, they must be expected to be of a lower degree of complication than the automaton itself"; furthermore, he described living organisms as follows [37]: "They produce new organisms with no decrease in complexity. In addition, there are long periods of evolution during which the complexity is even increasing." Our self-decomposable mortal automaton, having greater complexity, achieves prosperity of its offspring exceeding that of its immortal prototype. Consequently, we hypothesized that mortal organisms with altruistic self-decomposition emerge evolutionarily from immortal organisms [18, 22].

We constructed our experimental simulation model using SIVA configured with a terrestrial-type finite, heterogeneous environment. First, we designed an immortal organism as a precursor at an evolutionary stage just prior to its becoming a mortal organism, and configured a virtual ecosystem inhabited by that precursor organism as its only indigenous species. Then we considered what might become of a mortal individual with altruistic self-decomposition that had emerged evolutionarily from an indigenous immortal organism through mutation. As shown in Figures 3 and 4, the mortal VLIs consist of automata A, B, C, and FZ, each of which is a virtual protein with a specific function, as well as a data tape I_{D+FZ}, that is, a virtual genome that stores the data for each automaton. Automaton FZ, which has decomposed VLI itself, was activated whenever either of the following conditions was determined to be true: (1) the VLI encountered an environment incompatible with its survival, or (2) the VLI's life span had ended. For immortal VLIs, therefore, we assumed such conditions to be false so as not to make FZ execute self-decomposition. If this mechanism became the target of a mutation that canceled the "false" setting, it would correctly determine the above conditions to be true. Consequently, the mutant VLI could activate FZ and execute its own self-decomposition, resulting in the evolutionary emergence of a mortal organism with altruistic self-decomposition. We seeded an immortal VLI possessing this precursor of a genetic program for death at the very center habitation point of an ecosystem whose environmental conditions were deemed most suitable for a VLI.

The mutation rate was determined as follows. Existing terrestrial lives tend to have a higher mutation rate, as the length of their genome is shorter. For example, an organism with a genome of 10^4 molecules has a mutation rate 10^{-4}. Since the virtual genomes of VLIs in the present simulation consisted of 1,275 VM molecules, we used three mutation rates: 0.005, 0.002, and 0.001.

For each of the three mutation rates, we conducted 200 to 800 simulations of 800 passage durations each and observed changes in size of habitation area, number of VLIs, and frequency of mutation. Here one passage duration corresponded to 5 time counts (TCs, the unit of virtual time in SIVA-T05), because it took at least 5 TCs for a newborn individual to reproduce itself in our current simulation experiments. We have used the passage duration as the time unit in this report.

3 Results

Simulations of whether mortal VLIs emerging from immortal VLIs through mutation survived and proliferated obtained the following results.

Table 1 shows the proportions of the simulations in which mortal VLIs emerged and then survived at each mutation rate. Not so many mortal mutants emerged evolutionarily within the passage duration of 800, and, even when emerging, most of them were surpassed by native immortal VLIs and did not survive. The survival rates of emerging mortal mutants, however, were not negligible: 3.5%, 1.4%, and

Table 1. Proportions of the evolutionary emergence and survival of mortal virtual-life individuals.

Mutation rate	Total number of simulations	Proportion of emergence (number of simulations)	Proportion of survival (number of simulations)	Survival proportion of simulations with emergence
0.005	200	29% (58)	3.5% (7)	12%
0.002	500	11% (56)	1.4% (7)	13%
0.001	800	5.3% (42)	0.25% (2)	4.8%

Q4

0.25%, respectively, of the total number of simulations at mutation rates of 0.005, 0.002, and 0.001. Namely, the mortal mutants and their offspring that evolutionarily acquired altruistic self-decomposition were mostly but not altogether exterminated, with some surviving in extremely low proportions.

It was noteworthy that once the emerging mortal mutants survived and left offspring, these invariably surpassed immortal VLIs and increasingly proliferated as the passage duration became longer. Figure 5 shows changes in habitation area, number of VLIs, and frequency of mutation in a typical example for each of the three mutation rates, and indicates that the numbers of individuals and the frequency of mutation of mortal VLIs were quite low as they began to emerge, but, as their activity gradually increased, they began to surpass immortal VLIs between passage durations of 300 and 400, and afterwards proliferated exponentially.

4 Discussion

4.1 Emergence and Prosperity of Mortal Organisms

We carried out an evolutionary simulation experiment using our artificial ecosystem SIVA-T05, modeled for a finite, heterogeneous terrestrial environment and arranged in a biomolecular hierarchy. When a mortal mutant individual endowed with an evolutionarily acquired genetic program for death was born in a place in which immortal organisms already existed as indigenous ones, although

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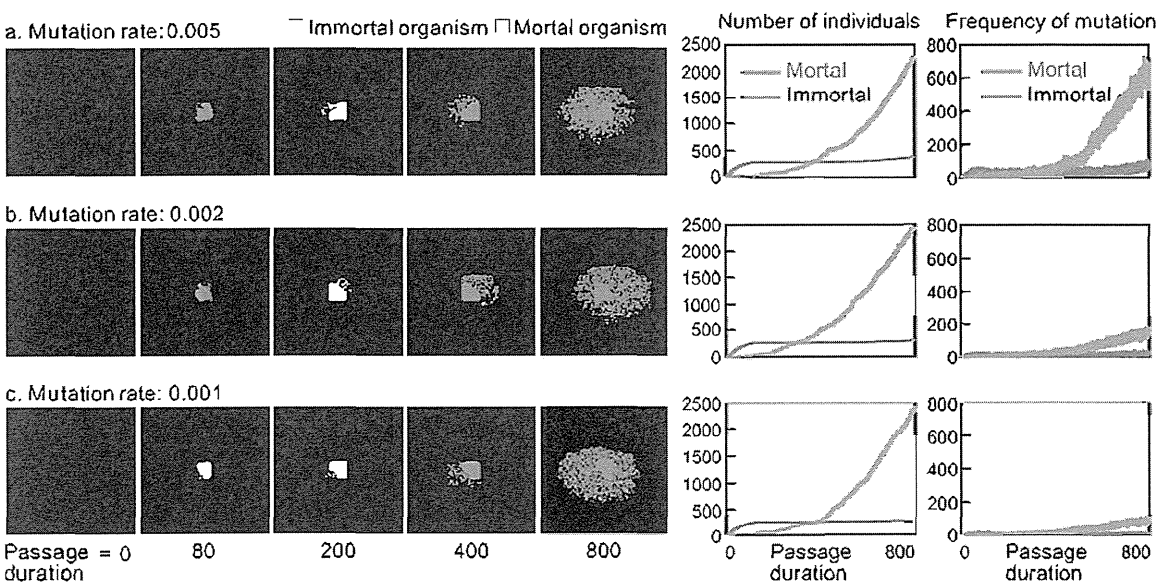

Figure 5. Successive changes in the distribution of individuals, the number of individuals, and the frequency of mutation of mortal and immortal VLIs when mortal VLIs emerged evolutionarily from immortal VLIs through mutation in the ecosystem where only immortal VLIs existed: (a) mutation rate 0.005; (b) mutation rate 0.002; (c) mutation rate 0.001. The mortal VLIs, which emerged and survived at a very low ratio, clearly surpassed immortal VLIs and became prosperous with adaptive divergence under various environmental conditions.

such mortal individuals had difficulty in surviving, yet they did survive and produced offspring, albeit at a very low reproductive rate. We also showed that, once these living individuals survived, they invariably prospered and surpassed existing immortal organisms.

Is it possible to apply such a finding to the actual evolutionary history of the terrestrial ecosystem? For example, since the evolution and prosperity of mortal organisms were only observed twice out of eight hundred simulations with a mutation rate of 0.001, such a result might be deemed merely an extremely rare phenomenon. However, in consideration of the enormously long evolutionary period of 3.8 billion years since most ancestors of life emerged, the boundless expanse of the terrestrial environment relative to a unicellular organism as minute as a few microns, for which even a cubic meter of water environment would be greatly expansive, and the immeasurable diversity and heterogeneity of matter and energy, we suggest that evolution from immortal to mortal life might well occur even if the probability might be lower than what we employed in the simulations.

4.2 Background to the Superiority of Mortal Organisms

As shown in our research, including the current experiment, the superiority of mortal organisms to immortal organisms pertains to the following background. Immortal organisms dominate space and materials, once secured, while the volume of resources in the ecosystem to sustain life activities monotonically decreases. With less chance of reproduction in association with a decrease of resources, the chances for mutation and evolutionary adaptation are reduced. By contrast, mortal organisms through self-decomposition release space and return their parts to the environment for other organisms to reutilize. As a result, accumulated mutations through the continuous alternation of generations accelerate evolutionary adaptation to the environment.

Death is the non-reversible termination of two definitive attributes of living individuals, namely, self-preservation and self-reproduction. In terrestrial lives, these attributes are associated with autolysis, which we have modeled as programmed death accompanied by altruistic self-decomposition, that is, the restoration of biomaterials and habitation space to the environment. In terrestrial lives, moreover, death is triggered not only by external forces such as predation, injury, or infection, but also by overwhelming environmental unconformity and the normal life span of that species. Consequently, death is essentially inevitable for terrestrial lives.

From the viewpoint of the individual-centric umwelt[1] considering the life principles of self-preservation and self-reproduction, such death of a terrestrial life form can be understood as the last defect of life, which should be essentially flawless, and as the epitome of the incompleteness of life, which could be overcome through evolution. Nonetheless, in our previous evolutionary simulation studies based on the ecosystem-dominant umwelt model, we found that living individuals endowed with a mortal genetic program that includes altruistic self-decomposition prospered and surpassed flawless living individuals with perfect self-preservation and self-reproduction in a heterogeneous, complex, terrestrial-type virtual environment.

The current experiment revealed that offspring of indigenous immortal organisms prospered more significantly than did their parents because of the autonomous, altruistic mortal genetic program newly installed in the immortal organisms that converted them into mortal organisms; that is, they evolutionarily acquired a paradoxical survival strategy. This finding encourages us to create a model for the acquisition of autonomous, altruistic death as the fruit of evolution.

Independent of the studies that we have undertaken since 1987 [9–11, 18–26], Peter M. Todd implemented artificial death in his ALife system [33, 34], and those experiments supported the recognition shared with us that death affords another entity its space in which to exist, and that death, accordingly, is essential throughout the ongoing evolutionary process. Nevertheless, the model of death constructed by Todd differs from ours in two obvious respects. First, death in Todd's model affords no process by which the organism might decompose itself into constituent parts for the efficient and collective reutilization of other organisms, which is an essential feature of our model. Second, the

1 The concept of *Umwelt* as proposed by Jakob von Uexküll [38].

death of an individual in Todd's model appears as a probabilistic phenomenon, or as a given event controlled by the simulation system, in sharp contrast to the activation of death in our model, which is an independent and autonomous process genetically regulated in the individual that starts from detection either of the end of its life span or of excess unconformity with the environment. Consequently, it would be difficult to rely on the ALife system as constructed by Todd to investigate the evolutionary emergence of death in a terrestrial ecosystem.

4.3 Reorganization of the Altruistic Concept

In this section, we describe the concept of altruism that informs our research, including the current study. In conventional zoology, altruism is defined, for example, as "a self-jeopardizing, self-exposing or self-sacrificing behaviour of animals, i.e. any unselfish behaviour pattern which increases the fitness of the object at expense of the altruistic individual" (*Elsevier's Dictionary of Biology* [4]). Thus, in the field of zoology, both a negative effect on a self-individual (i.e., sacrifice) and a positive effect on other individuals (i.e., contribution) are usually regarded as an intrinsic attribute of altruism. Yet there remains the need to measure the degree of altruism in terms of the magnitude of sacrifice. On the other hand, when we look at how altruism is described as a more general concept, we find that the *Oxford English Dictionary* [27] calls it "Devotion to the welfare of others, regard for others, as a principle of action." This notion offers a slightly different slant on altruism by referring to the target of altruistic behaviors as not likely limited to an individual and with greater attention paid to the contribution per se rather than to the sacrifice itself. There seems to be an inconsistency between the zoological concept and the general concept of altruism as to whether the following two conditions should be regarded as essential attributes of altruism: targeting only individuals and always accompanying sacrifice. This semantic deviation, for example, poses a challenging question: Which is more altruistic, a tremendous
Q5 contribution with negligible sacrifice or an tremendous sacrifice with negligible contribution?

In order to adequately address such a question in setting up a reliable framework for our own research, we examined the conventional concept of altruism, which is constrained by individuals and sacrifice. In doing so, we reconstructed a more flexible concept of altruism [25, 26], which need not restrict its target to an individual and need not regard sacrifice as an indispensable requirement.

We redefined an altruistic phenomenon as a phenomenon by which a living individual renders certain biological benefits to a part of the ecosystem including individuals as well as to that ecosystem as a whole, regardless of any biological benefit or disadvantage to itself [25, 26], and we have deployed this construction as a conceptual apparatus in our research. The key point is not to regard sacrifice as an indispensable requirement and not to restrict recipients of contribution to individuals, while extending the notion of recipient to the system level, that is, the ecosystem itself. The biological benefits for the ecosystem may cover various aspects, including the restoration of the environment to its original state, regeneration of reproductive potential, mutation increase, biodiversity, and so forth.

The problem of deciding whether to focus on sacrifice or contribution in altruism or whether to restrict altruism to an individual or extend it to a system is similar to deciding whether to consider light as a wave or a particle in physics. It is well known that a feature of light is easier to understand as a wave in some cases, and as a particle in other cases, and those two aspects are practically selected depending on the circumstances. Such a relationship between two standpoints may correspond to the interrelationship of paradigms, or independent axiomatic systems not mutually constraining, as proposed by Thomas Kuhn [7].

4.4 Evolutionary Mechanism of Altruistic Phenomena

Many theories and principles have been proposed and examined with regard to the evolution of altruism. Kin selection mechanisms based on inclusive fitness, first proposed by John B. S. Haldane and refined upon by William D. Hamilton and George R. Price [5, 6, 28], elegantly explains the evolution of various altruistic behaviors in animals. In addition, related studies by Tom Lenaerts, Francisco C. Santos, and Jorge M. Pacheco [30, 31] suggest that heterogeneity and complexity, which are essential attributes of the real world, have led to the evolutionary emergence and sustainability of

cooperation, a concept closely related to that of altruism. Such studies are compatible and complementary with our finding that mortal organisms endowed with altruism overwhelmingly proliferate in a complex heterogeneous terrestrial-type environment.

In addition, Martin A. Nowak has provided a useful framework to explain the evolution of cooperation—which is closely related to the concept of altruism—on the basis of the principle of natural selection [13–16, 35]. Although Nowak's idea appears to be mainly based on an interest in the origin and evolution of cooperative behaviors in humans and therefore may not precisely correspond to our concept of altruism, his framework indeed effectively explains the PSD mechanism.

According to Nowak, mechanisms for the evolution of cooperation can be classified according to five rules according to the state of the interaction among components, as follows: (i) kin selection, (ii) direct reciprocity, (iii) indirect reciprocity, (iv) network reciprocity, and (v) group selection [14]. In summary, the evolution of cooperation may occur in the following manner: (i) Kin selection operates when the donor and the recipient of an altruistic act are genetic relatives. (ii) Direct reciprocity operates when there are repeated encounters between the same two individuals and both remember their history of cooperation. (iii) Indirect reciprocity is based on reputation, that is, a helpful individual is more likely to receive help. (iv) Network reciprocity operates when cooperators form a close network in which reciprocity develops. (v) Group selection involves the recognition that competition is not only between individuals but also among groups. Group selection sometimes has priority over individual selection, and pure cooperator groups prevail over groups laden with defectors.

Nowak examined this taxonomy while referring to a large number of previous studies, pointing out that emerging cooperation evolves from natural selection as appropriate conditions are satisfied, whereas it becomes extinct when such conditions are not satisfied. He also formulated five simple rules, dependent mainly on the parameters of cost and benefit, by which to discriminate whether or not cooperation will be naturally selected [14]. Based on this framework, he insightfully suggested natural cooperation as a third principle of evolution besides mutation and natural selection.

Nowak's insight with regard to the mechanisms for the evolution of cooperation throws light on our findings of a paradoxical phenomenon in which individuals actualizing altruistic death by means of self-decomposition, though seemingly withdrawing from the struggle for survival, actually prevail over immortal individuals. Needless to say, the kin selection mechanism at least partly explains our seemingly contradictory finding that a species endowed with altruistic death is superior to one without it. Moreover, network reciprocity may also work because the environmental conditions of our simulation space were heterogeneously determined and the virtual individuals were set so that they could not migrate outside their habitation area. Therefore, each group of virtual individuals adapted evolutionarily to environmental conditions specific to the habitat spatial block, and the individuals were spatially separated from other individuals existing under other environmental conditions. Since these circumstances can be interpreted as indicating that virtual individuals within each spatial block form a close network, the superiority of PSD may include a mechanism categorized as network reciprocity. In addition, because a network constructed only by mortal lives reuses materials and space more efficiently than does that inclusive of immortal lives, it likely accelerates evolutionary adaptation to the environment. Thus group selection is also of importance. Moreover, multiple evolutionary mechanisms interacting with one another could be involved.

Thus Nowak's five rules for the evolution of cooperation convincingly account for at least a part of the PSD mechanism. Yet there one more point that should be considered. That is, is Nowak's model sufficiently robust that we can exclude an examination of unknown factors possibly missing from his model?

First, we need to mention that, in our experimental system, all VLIs in the ecosystem are offspring of one initially seeded VLI, which means that all VLIs participating in one simulation are genetic relatives. In particular, since the initial mortal VLI was born from an immortal VLI through a point mutation, these two VLIs share the same genes to a significant degree. At the same time, mortal VLIs have advanced through evolution to other spatial blocks having remarkably different environmental conditions from those of the spatial block in which the initial VLIs existed. This suggests that the genotypes of mortal VLIs distant from the initial habitat point should differ markedly from that of

the initial mortal VLI, due to repeated mutations. Thus the commonality of genotypes between different VLIs, that is, the degree of kin relationship, would continuously decrease. It follows that an evolutionary force driven by kin selection cannot be strengthened, although it can be weakened, as time passes. In our simulation experiments, however, the number of mutations kept increasing and evolutionary adaptation to the environment continuously accelerated. It is necessary to examine whether the kin selection mechanism, basically resting its claim on the commonality between genotypes, can be independently verified as a determinant force for evolution in such situations.

Second, in our simulations it was noteworthy that a VLI contributing to others through altruistic self-decomposition disappeared in tandem with the self-decomposition process and thus no longer existed as a target for reciprocity or retribution nor as a subject of recognition and decision. Therefore, it is difficult to compare the degree of risk or sacrifice with the degree of benefit at the individual level according to Nowak's model. We should carefully consider how altruistic phenomena might be actively selected under such a condition.

Third, in order to delve into potential activities within our ecosystem-dominant umwelt model, the SIVA simulator used in our research was designed to eliminate direct interactions between particular individuals such as bestowal, partnership, predation, and competition as well as sexual reproduction. In our experimental system, each VLI is designed to interact only with its environment through utilizing materials for self-reproduction, releasing its decomposed parts at self-decomposition, and occupying and releasing habitat space, as shown in Figure 3. By assumption, mutual direct interaction between or among plural VLIs cannot occur. Instead, all interaction between VLIs always occurs indirectly through the ecosystem as a change in environmental conditions. No decomposed parts produced by the PSD mechanism belong to any particular individuals, but they do exist as a group of environmental materials that can be utilized by any VLI in the ecosystem. Decomposed parts are utilized by any VLI, and direct descendants are not accorded any special favoritism. In other words, the recipients of decomposed biomaterial contributed by self-decomposition are not restricted to any particular individual or group of individuals. In order to actualize such a concept, we did not install any mechanisms into the VLIs by which to recognize and discriminate other individuals. Thus, using SIVA, we can perform a simulation experiment in which particular individuals do not become targets for cooperation or antagonism based on individual discrimination. All our past simulations were conducted in such a way. Our experimental setting attempts to draw a distinction counter to the conventional meaning of "selection" implied by individual discrimination, which can be regarded as a selection function regulated by internal factors. Selections by individual discrimination should be kept distinct from natural selections induced by an external factor of environmental conditions.

Given these considerations, we implemented for the SIVA simulator a most primitive theoretical model for life in which the fundamental principle of terrestrial life is ultimately simplified and abstracted, taking von Neumann's self-reproductive automaton [37] as our prototype, that is, it reproduces and decomposes itself solely according to genetic information. All biological information possessed by a VLI is remembered as a sequence of virtual genomes while precluding any other method by which to remember information. In other words, in our experimental system, a VLI does not make use of any method by which to remember experiences during its life span or to discriminate between individuals, and all its life activities are regulated solely according to genetic information in read-only memory (that is, a genome), and can be changed only by mutation in the event of self-reproduction of VLIs.

Taking into account the above-mentioned points raised by our experimental system, we reexamined Nowak's five rules from the viewpoint of evolutionary biology, especially focusing on evolutional development of a biological control system, and discussed their bearing on our studies.

First of all, we focused on the most developed biological control system of interest to Nowak, the central nervous system in vertebrates (including humans), in which plasticity of efficiency of information transmission at the synaptic junction and its extensive accumulation effectively function to remember experiences [1]. Functions of individual discrimination and behavior selection based on such a flexible memory function are not only essential for indirect reciprocity requiring communication regarding others' reputation, but are also important for direct reciprocity requiring contribution to

specific individuals that have contributed to oneself, as well as for network reciprocity requiring clustering with specific individuals. The distributed neural network connecting neural ganglia, which corresponds to the biological control system evolutionarily preceding the central nervous system, is fundamental to direct reciprocity and network reciprocity based on individual discrimination of, for example, social insects. Of course, such functions of the nervous system can enable organisms to actualize group selection and kin selection.

Next, the biological control system evolutionarily preceding the nervous systems is a chemical messenger, typically observed as the endocrine system of animals, hormones of multicellular plants, pheromones of insects, and so forth. In the case of organisms without a nervous system, selective expression of life activities based on individual discrimination by means of a certain chemical messenger system is indispensable for group selection, in which cooperative behaviors should be exerted on discriminating individuals within a group from those outside the group, and for kin selection, in which cooperative behaviors should be according to the degree of kinship. Nowak's five rules essentially require reference to remembered information to discriminate whether other individuals are appropriate recipients of cooperation, and to selectively express a cooperative behavior toward a specific individual or a group of individuals (including the species) under the commitment of a relatively developed biological control system, as mentioned above.

On the other hand, there is a biological control mechanism evolutionarily preceding the chemical messenger system, namely, metabolic regulation on the basis of genetic information remembered in DNA a priori. This mechanism can be actualized by a single cell alone, which is a basic unit of terrestrial life, and therefore it is fundamental to all kinds of biological control systems of terrestrial lives. Our SIVA simulator is designed to focus on evolutionary phenomena that can be actualized solely by means of this biological control system. Thus the PSD mechanism corresponds to the most primitive class of terrestrial lives, namely, cells controlled only by metabolic regulation, and does not reach any level of the five mechanisms as proposed by Nowak. Terrestrial lives in this class not only control themselves, but also support those belonging to higher-developed classes, of which Nowak took note, possibly as an indispensable infrastructure. It is noteworthy that PSD, which is an altruistic phenomenon specific to metabolic regulation or autolysis, may exist universally in terrestrial lives, as suggested by the universal extent of lysosomes (i.e., organelles executing PSD) in every eukaryote cell.

Various findings from our experiments support Nowak's idea that "we might add natural cooperation as a third fundamental principle of evolution beside mutation and natural selection." Nowak incorporated evolutionary mechanisms of cooperative behavior in higher organisms, which are dependent on interaction between particular living individuals. Such mechanisms require biological control systems, such as chemical messengers and, ideally, a central nervous system, that enable the remembering of experience and the ability to discriminate between individuals. In other words, functions to discriminate and select other individuals, partly through referring to remembered information, are inseparable from cooperative behaviors. Consequently, Nowak's framework quite adequately accounts for cooperative behaviors, which can be regarded as a certain type of altruistic phenomena that target only specific individuals or groups.

By contrast, our research has suggested that the involvement of altruism promotes evolutionary adaptation even in very primitive life forms equipped with only the fundamental principle of terrestrial life, namely, self-reproduction and self-decomposition regulated solely by a genetic program, and without any functions by which to discriminate between individuals. This means that altruistic phenomena can be an evolutionary force even without requiring discrimination and selection of other living individuals, that is, interaction including cooperation among specific individuals.

An individual-oriented evolutionary mechanism is conventionally accepted in which a living individual or the population to which it belongs actively retains its specific properties based on interaction between individuals, that is, discrimination and selection of other individuals, as is the case with higher animals. On the other hand, in our experimental conditions it was difficult for such individual-oriented discrimination and selection to occur. Nevertheless, the altruistic gene contributed to either a part of or the entire system through the restoration of the ecosystem to its original state in a way that rendered neither advantage nor disadvantage to individuals. As a result, the altruistic gene was naturally selected

as a trait suitable for such an environment. Our findings suggest the possibility of the existence of an ecosystem-oriented evolutionary mechanism. Yet it seems premature to conclude that no mechanism promoting evolution other than an individual-oriented mechanism exists. We therefore wish to pay increased attention to the possibility that a more fundamental ecosystem-oriented evolutionary mechanism of programmed death accompanied by altruistic self-decomposition could exist as the infrastructure of individual-oriented altruistic mechanisms.

4.5 Conclusion

As described above, our research has focused on a lacuna in previous research on altruism by paying keen attention to death with altruistic self-decomposition, which seemingly contradicts our understanding of the survival of the fittest and the struggle for existence. We have identified a mechanism by which mortal organisms through altruistic activity overcome immortal organisms deprived of altruism and prosper. We also suggest that such activity might promote evolution in a finite, heterogeneous terrestrial-type environment. In showing that the altruistic mortal gene endowed with self-decomposition can be acquired through the evolution of immortal lives, our findings thus offer new insight into the overwhelming evolutionary superiority of lives with the altruistic mortal gene over immortal lives. We believe that such results might augur the opening of a new field of inquiry not only in the study of altruism, but also, more broadly, in the realm of evolutionary biology.

In addition, our present study advocates the study of artificial life based on artificial chemistry to simulate existent terrestrial life, as a promising research tool in the field of interdisciplinary life science including evolutionary biology. We believe in the potential of artificial chemistry in this regard and expect that the concepts, insights, and cutting-edge techniques of artificial life research will henceforward become ever more widely disseminated in all fields of inquiry related to life phenomena.

Acknowledgments

We wish to thank Dr. Katsunori Shimohara, Doshisha University, and Dr. Hideaki Suzuki, National Institute of Information and Communications Technology, for their valuable comments on our study; Dr. Hiroki Sayama, Binghamton University, for his contribution to the development of the earlier version of the SIVA series; Dr. Michael E. Workman and Ms. Jane S. Workman at BEST Ltd., Tokyo, for their patient editing; and members of Yamashiro Institute of Science and Culture for much appreciated technical support.

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Journal of Psychiatric Research 46 (2012) 905–912

Contents lists available at SciVerse ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Frontal and right temporal activations correlate negatively with depression severity during verbal fluency task: A multi-channel near-infrared spectroscopy study

Takamasa Noda [a,b,*], Sumiko Yoshida [a], Taro Matsuda [a], Nagahisa Okamoto [a], Kota Sakamoto [a], Shunsuke Koseki [c], Yotaro Numachi [a], Eisuke Matsushima [b], Hiroshi Kunugi [d], Teruhiko Higuchi [e]

[a] Department of Psychiatry, National Center of Neurology and Psychiatry Hospital, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187-8551, Japan
[b] Section of Liaison Psychiatry & Palliative Medicine Division of Comprehensive Patient Care, Graduate School of Medical & Dental Sciences, Tokyo Medical & Dental University, 1-5-45, Yushima, Bunkyo, Tokyo 113-8519, Japan
[c] Department of School Education, Aichi University of Education, 1, Hirosawa, Igayacho, Kariya, Aichi 448-8542, Japan
[d] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187-8502, Japan
[e] National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187-8551, Japan

ARTICLE INFO

Article history:
Received 2 November 2011
Received in revised form
14 March 2012
Accepted 2 April 2012

Keywords:
Hamilton Rating Scale for Depression
Major depressive disorder
Near-infrared spectroscopy
Severity of depression
Verbal fluency task

ABSTRACT

Multi-channel near-infrared spectroscopy (NIRS) is a noninvasive, on-the-spot, functional neuroimaging technique allowing detection of the spatiotemporal characteristics of brain activity. Previous NIRS studies indicated the oxy-hemoglobin (oxy-Hb) increase during a verbal fluency task (VFT) is attenuated in patients with major depressive disorder (MDD) as compared with healthy controls. However, the possible relationship between depression symptom severity and oxy-Hb change on NIRS has not yet been elucidated. To examine this relationship, we recruited 30 patients with MDD and 30 age-, gender- and intelligence quotient-matched controls. All underwent NIRS during VFT. As expected, the oxy-Hb increase during the task was significantly smaller in patients than in controls. After false discovery rate correction using 31 channels, the mean increase in oxy-Hb during the task showed a significant negative correlation with the total score of the Hamilton Rating Scale for Depression 21-item version (ch25: rho = −.56; FDR-corrected p: .001). When each item of the HAM-D21 was examined individually, insomnia early in 9 channels (rho = −.63 to −.46; FDR corrected p: .000–.014), work and activity in 2 channels (rho = −.61 to −.57; FDR corrected p: .001 to .003) and psychomotor retardation in 12 channels (rho = −.70 to −.44; FDR corrected p: .000–.018) showed significant negative correlations with the mean oxy-Hb increase in the right frontal temporal region. Although it is possible that our results were affected by medication, these data suggest reduced right frontal temporal activation on NIRS during VFT is related to the symptom severity of MDD.

1. Introduction

Major depressive disorder (MDD) is a severe and common psychiatric disorder with a lifetime prevalence of 6.7 per 100 (Waraich et al., 2004). Although depressive symptoms per se do not specifically appear in MDD but also in other psychiatric disorders including bipolar disorders, we do not have an objective diagnostic marker to obtain a clear-cut diagnosis for those patients. In Japan, a relatively new neuroimaging method, near-infrared spectroscopy (NIRS) has been approved by the Ministry of Health, Labor and Welfare as a highly advanced medical technology to help distinguish between schizophrenia, depression and bipolar disorders in 2009. Verbal fluency task (VFT) is recommended as an activation task because of a relatively rich store of data. VFT is an easy task to examine the executive function and frequently used in neuroimaging studies (Alvarez and Emory, 2006) and is known to activate prefrontal cortex (PFC) in healthy subjects (Frith et al., 1991; Schlösser et al., 1998). Numerous neuropsychological studies suggest that patients with MDD show executive dysfunction (Gohier et al., 2009; Rose and Ebmeier, 2006; Fossati et al., 2003; Porter et al., 2003; Degl'Innocenti et al., 1998).

Multi-channel near-infrared spectroscopy (NIRS) is a noninvasive, on-the-spot, restraint-free functional neuroimaging technique allowing detection of the spatiotemporal characteristics of brain

* Corresponding author. Department of Psychiatry, National Center of Neurology and Psychiatry Hospital, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187-8551, Japan. Tel.: +81 42 341 2711; fax: +81 42 346 1705.
E-mail address: t-noda@ncnp.go.jp (T. Noda).

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doi:10.1016/j.jpsychires.2012.04.001

function near the brain surface using near-infrared light (Strangman et al., 2002a; Boas et al., 2004). NIRS has enabled bedside measurement of the concentrations of oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb) changes with a high time resolution (.1 s). The concentrations of oxy-Hb and deoxy-Hb are assumed to reflect the regional cerebral blood volume (rCBV) changes, which was supported by the simultaneous NIRS and PET study (Villringer et al., 1997; Ohmae et al., 2006).

In fact, numerous studies have demonstrated that the oxy-Hb increase in the fronto-temporal regions during a VFT is significantly smaller in patients with MDD than in those with bipolar disorder or healthy controls (Pu et al., 2008; Kameyama et al., 2006; Suto et al., 2004; Matsuo et al., 2002). Moreover, NIRS studies using VFT have also demonstrated frontal lobe dysfunction in schizophrenia (Suto et al., 2004; Takizawa et al., 2008), and panic disorder (Nishimura et al., 2007). However, the relationship between depression symptom severity at the time of examination and oxy-Hb change on NIRS has not yet been clarified.

In neuroimaging studies using other methodologies, focusing on cortex level that NIRS reflects, positron emission tomography (PET) studies found that abnormal reductions of cerebral blood flow (CBF) and metabolism in patients with MDD in PFC (Kimbrell et al., 2002; Bench et al., 1995; Mayberg et al., 1994; Baxter et al., 1989). As for the relationship between executive function and CBF or metabolism, Elliott et al. (1997) showed activation in PFC was significantly attenuated relative to controls during the Tower of London planning task in PET study. In a functional magnetic resonance imaging (fMRI) study, depressed patients showed significant decreased prefrontal activation during VFT (Okada et al., 2003).

As for the relationship between depression symptom severity and frontal lobe function, Brody et al. (1999) found a positive correlation between change in Hamilton Rating Scale for Depression (HAM-D) scores and change in normalized inferior frontal gyrus (IFG) and ventrolateral PFC (VLPFC) metabolism, which indicates that IFG metabolism increased and VLPFC metabolism decreased as depression symptoms became better. Other initial studies also suggest that abnormal functions in dorsolateral PFC (DLPFC) are mood state dependent, attenuated during the depressed mood and reversing during symptom remission (Bench et al., 1995; Mayberg et al., 1994). In contrast, Drevets et al. (2002) showed the persistence of abnormal metabolic deficits using PET measures in the dorsomedial/dorsal anterolateral PFC in MDD during treatment. According to a review by Drevets (2000), a complex relationship exists between depression symptom severity and metabolic activity in the orbital cortex and VLPFC.

Findings obtained by more recent studies investigating cross-sectional relationship between depression symptom severity and brain function assessed by basal regional CBF and metabolism are also inconsistent. For example, Périco et al. (2005) reported that depression symptom severity was negatively correlated with regional CBF (rCBF) in the left amygdala, lentiform nucleus, and parahippocampal gyrus, and positively correlated with rCBF in the right postero-lateral parietal cortex, whereas Milak et al. (2005) showed only positive correlations in bilateral mesiotemporal cortex, parts of the ventral subgenual basal forebrain, and most of the thalamus, hypothalamus, ventral striatum, and midbrain. Accordingly more studies are warranted to clarify the relationship between depression severity and brain activity including frontal lobe function.

In the present study, considering the consistent finding of attenuated oxy-Hb changes during VFT in the fronto-temporal regions in depression, we hypothesized that oxy-Hb changes during VFT in NIRS could be objective indicators of depressive symptom severity. Thus, we used multi-channel NIRS to investigate the relationship between oxy-Hb changes and symptom severity in patients with MDD. Because NIRS can be measured easily and noninvasively in a restraint-free environment over a short amount of time we expect that NIRS can be widely used to assess objectively depressive symptom severity as a clinical examination.

2. Materials and methods

2.1. Subjects

The subjects were 30 patients with MDD, and 30 healthy volunteers matched for age, gender and premorbid intelligence quotient (IQ). Premorbid IQ was estimated using the Japanese version of the National Adult Reading Test (Matsuoka et al., 2006). All subjects were right-handed according to the Edinburgh Inventory (Oldfield, 1971) and were native speakers of Japanese. All MDD subjects were outpatients of the National Center of Neurology and Psychiatry Hospital in Tokyo, Japan. They were diagnosed according to the Structured Clinical Interview for the Diagnostic Statistical Manual of Mental Disorders, 4th edition (DSM-IV) Axis I Disorders (SCID-I; First et al., 1995) by experienced psychiatrists. All patients were medicated with antidepressants. Twenty-seven out of 30 patients were prescribed with one or two antidepressants, 16 with SSRIs, 12 with tricyclics, 7 with milnacipran, 5 with tetracyclics, 2 with trazodone and 1 with mirtazapine. In addition, 20 patients were prescribed with anxiolytics, 16 with hypnotics, 7 with mood stabilizers and 9 with antipsychotics (Supplementary Table 1). Daily doses of all antidepressants were converted to an equivalent dose of imipramine (Inagaki and Inada, 2006) and anxiolytics/hypnotics to that of diazepam (Inagaki and Inada, 2006) for each patient. The controls were healthy volunteers recruited from the same geographical area through advertisements in free local magazines and our website announcement. They were interviewed using the SCID-I for MDD or SCID-NP for healthy volunteers and an unstructured interview for family history, and those individuals who had a current or past history of Axis I psychiatric disorder or a positive family history of Axis I psychiatric disorder within their first degree relatives were excluded. The exclusion criteria for both groups were previous head trauma, neurological illness, a history of electroconvulsive therapy, alcohol/substance abuse or addiction.

After the study procedures had been fully explained, written informed consent was obtained from every participant. This study was approved by the ethics committee of the National Center of Neurology and Psychiatry.

2.2. Clinical assessment

Depressive symptoms and the level of social functioning were evaluated by a single experienced psychiatrist using the GRID Hamilton Rating Scale for Depression 21-item version (GRID HAM-D21; Kalali et al., 2002) and Global Assessment of Functioning scores (GAF; American Psychiatric Association, 1994), respectively, without knowledge of the NIRS data on the same day that the NIRS measurements were conducted. Sleepiness was evaluated as the score on the Stanford Sleepiness Scale (SSS; Hoddes et al., 1973).

2.3. Activation task

The activation task was a letter version of VFT similar to that described by Takizawa et al. (2008). During the VFT, changes in oxy-Hb and deoxy-Hb were measured. The VFT consisted of a 30-sec pre-task baseline, a 60-sec VFT, and a 70-sec post-task baseline. The subjects were instructed to repeat the syllables /a/, /i/, /u/, /e/ and /o/ during the pre-task and post-task baseline periods. For the VFT, the subjects were instructed to generate as many words as possible.

One of the three initial syllables (A; 0–20 s /a/, /to/, or /na/, B; 20–40 s /i/, /ki/, or /se/, C; 40–60 s /o/, /ta/, or /ha/) was randomly

presented on the computer display placed in front of the subjects, every 20 s during the 60-sec task. The number of possible combinations of syllables is 27 (A:3 × B:3 × C:3 = 27). We adopted 15 among the possible combinations. The number of correct words generated during the task was determined as a measure of task performance.

3. NIRS measurements

3.1. NIRS device

We used a 52-channels NIRS (ETG-4000 Optical Topography System; Hitachi Medical Co., Tokyo, Japan) which measures relative changes in oxy-Hb and deoxy-Hb using two wavelengths (695 nm and 830 nm) of infrared light based on the modified Beer–Lambert law (Yamashita et al., 1996). With this system, these Hb values include a differential pathlength factor (DPF). In the NIRS system, "hemoglobin concentration change*DPF" is calculated as a solution to the simultaneous equations based on the Beer–Lambert law, which cannot escape the effect of DPF. Although DPF varies among various brain regions Zhao et al., using a Monte Carlo simulation, reported the estimated DPF variation in the forehead region of adult humans was roughly homogeneous (Zhao et al., 2002).

The distance between a pair of source-detector probes was set at 3.0 cm and each area measured between a pair of source-detector probes was defined as a 'channel'. The NIRS device is considered to measure 'channels' at a 2–3 cm depth from the scalp, that is, at the surface of the cerebral cortex (Hock et al., 1997; Okada and Delpy, 2003, Toronov et al., 2001).

3.2. Probe positioning and measurement points

The NIRS probes were fixed with 3 × 11 thermoplastic shells, with the lowest probes positioned along the Fp1–Fp2 line according to the international 10–20 system used in electroencephalography. The probes can measure Hb values from bilateral prefrontal and temporal surface regions. The measuring points were labeled ch1 to ch52 from right-posterior to left-anterior (Fig. 1). The correspondence between these NIRS channels and the measurement points on the cerebral cortex was confirmed by a multi-subject study of anatomical cranio-cerebral correlations (Okamoto et al., 2004) and presented on the basis of results obtained by the virtual registration method (Tsuzuki et al., 2007).

3.3. Measurement parameters

The rate of data sampling was .1 second (s). The obtained data were analyzed using integral mode; the pre-task baseline was determined as the mean over a 10 s period just prior to the task period, and the post-task baseline was determined as the mean over the last 5 s of the post-task period. Linear fitting was then applied to the data between these two baselines. The moving average method using a window width of 5 s was applied to remove any short-term motion artifacts. Because we could not remove all artifacts in this way, we applied automatic rejection of data with artifacts separately for each channel (Takizawa et al., 2008).

According to the aforementioned measurement parameters for integral mode, the waveforms of oxy-Hb, deoxy-Hb and total-Hb

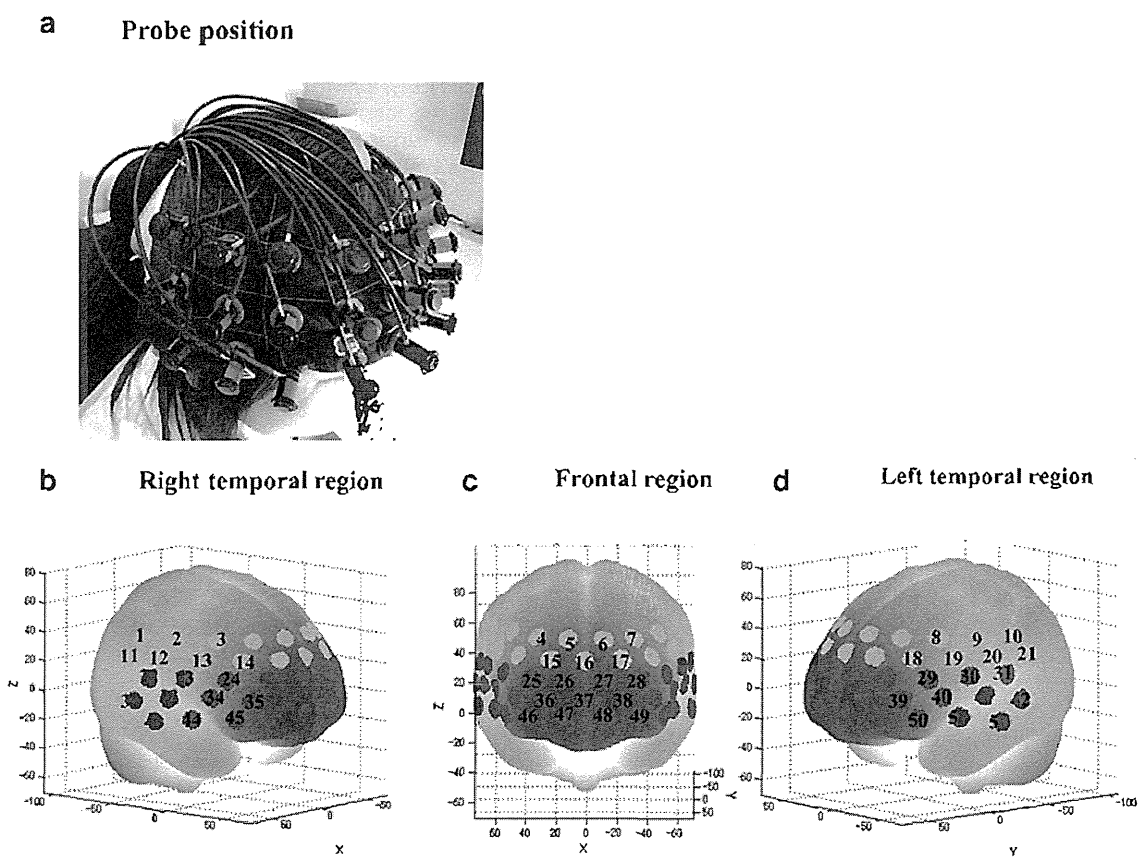

Fig. 1. Measurement points of 52 channels for near-infrared spectroscopy (NIRS) (a) Probes with 3 × 11 thermoplastic shells were placed over a subject's bilateral frontal regions. (b–d) The 52 measuring positions of the NIRS device are superimposed on the 3D-reconstructed cerebral surface, based on magnetic resonance imaging. The 52 measuring positions are labeled ch1 to ch52, from the right posterior to the left posterior. The dimensional figures b, c and d indicate the right temporal, frontal and left temporal brain regions, respectively. Because acquired NIRS data from the 21 channels in the upper two rows (pink channels) clearly contained artifacts presumably due to hair, as indicated by visual inspection of the waveforms, and signal to noise ratio seemed to be low, they were excluded from statistical analyses.

changes were acquired from each subject in all 52 channels during VFT.

3.4. Measurement environment

The subjects sat on a comfortable chair in a silent and day-lit room. They were instructed to minimize motions such as head movements, strong biting and blinking during the NIRS measurement, to avoid artifacts.

Data clearly containing motion artifacts, based on both our observations and the NIRS recording, were excluded from further analyses.

4. Statistical analysis

Because acquired NIRS data from the 21 channels in the upper two rows clearly contained artifacts presumably due to hair, as indicated by visual inspection of the waveforms, and signal to noise ration seemed to be low, they were excluded from statistical analyses.

The χ^2 test or Student's t-test was used to compare proportions and means, respectively, between the MDD and control groups.

As for the analysis of the NIRS data, we focused on oxy-Hb data, since oxy-Hb change (task period − pre- and post-task baseline period) is assumed to more directly reflect cognitive activation than deoxy-Hb change as shown by a stronger correlation with blood-oxygenation level-dependent signal measured by fMRI (Strangman et al., 2002b). The mean oxy-Hb changes were compared between the two groups (MDD and control) for each channel using Student's t-test. To examine the relationships between oxy-Hb changes and HAM-D21 total scores, HAM-D21 subscale scores, GAF, or other clinical variables, Spearman's rhos were calculated for MDD patients.

All statistical analyses were performed using SPSS for Windows, version 18.0.0 software (SPSS Japan, Tokyo, Japan). A value of $p < .05$ (two-tailed) was considered to be statistically significant. We set the value of q specifying the maximum false discovery rate (FDR) at .05, such that the false positive rate was no more than 5% on average in treating the oxy-Hb data obtained from multiple channels (Singh and Dan, 2006).

5. Results

5.1. Demographic and clinical data of patients and controls

Table 1 summarizes demographic characteristics of the patients and controls. The two groups did not differ significantly in age, gender, handedness, estimated premorbid IQ or SSS.

5.2. Task performance

The number of words generated did not differ significantly among the 15 combinations employed (15 combinations: $F[1, 45] = 1.1$, $p = .39$; three initial syllables: $F[2, 90] = 1.2$, $p = .31$) in either group. The number of generated words during VFT did not differ significantly (patients: 12.3 ± 3.9; controls 13.9 ± 4.3, $t = 1.5$, $df = 58$, $p = .13$) between the MDD and control groups.

5.3. Group comparison

As shown in Fig. 2, the MDD group had significantly smaller oxy-Hb increases than the control group in 22 channels (ch22–29, ch32–33, ch35–39 and ch44-50; FDR-corrected p: .000–.024) during VFT.

5.4. Relationship with symptom severity at the time of examination

As shown in Fig. 2, there were significant negative correlations between mean oxy-Hb changes during the task and HAM-D21 total scores in one channel (ch25: rho = −.56; FDR-corrected p: .001). Mean oxy-Hb changes during the task period showed significant negative correlations with three individual items of the HAM-D21 subscale scores (Fig. 3); insomnia early in 9 channels (ch23, ch25–27, ch36–37 and ch46–48: rho = −.63 to −.46; FDR corrected p: .000–.014), work and activity in 2 channels (ch44 and ch45: rho = −.61 to −.57; FDR corrected p: .001 to .003), and psychomotor retardation in 12 channels (ch22–24, ch32, ch35–36, ch41, ch43–ch45, ch47 and ch51: rho = −.70 to −.44; FDR corrected p: .000–.018). Mean oxy-Hb changes showed no significant correlations with the remaining HAM-D21 subscale scores (i.e., depressed mood, guilt, insomnia middle, insomnia late, psychomotor agitation, anxiety psychic, anxiety somatic, loss of appetite, somatic symptoms general, sexual interest, hypochondriasis, loss of weight, insight, diurnal variation, and obsessional symptoms;) (Fig. 4).

Furthermore, mean oxy-Hb changes showed no significant correlation with task performance during VFT or other clinical variables, such as age, duration of illness, and sleepiness (data not shown).

5.5. Relationships with medication

There were no significant correlations between the HAM-D21 total score and doses of antidepressants (rho = −.23, $p = .22$) or anxiolytics (rho = .25, $p = .18$). There were significant negative correlations between mean oxy-Hb changes during the task and doses of antidepressants in 6 channels (ch31, ch40–41, ch45, ch50–51; rho = −.57

Table 1
Demographic and clinical data of patients with major depressive disorder and controls.

Demographics	Patients with depression ($n = 30$)	Healthy controls ($n = 30$)	Group difference p-value
Age (years)	36.7 ± 11.6	35.1 ± 9.4	.871
Gender (female/male)	16/14	16/14	1.000
Edinburgh handedness inventory (%)	92.9 ± 9.7	92.0 ± 11.5	.753
Age at onset (years)	30.9 ± 10.8	–	–
Duration of illness (years)	5.8 ± 4.1	–	–
Duration of medication (years)	5.0 ± 3.6	–	–
GRID HAM-D21 total score	16.7 ± 4.8	–	–
Estimated premorbid IQ	105.7 ± 9.5	105.9 ± 8.3	.953
Sleepiness	3.3 ± 1.1	2.9 ± .9	.104
GAF	57.6 ± 9.3		
Medication			
Imipramine equivalent dose (mg/day)	141.9 ± 127.6	–	–
Diazepam equivalent dose (mg/day)	8.5 ± 11.6	–	–

The χ^2 test or t-test was used to compare these variables between patients and controls. GAF, Global Assessment of Functioning; GRID HAM-D21, GRID Hamilton Rating Scale for Depression 21 item; IQ, Intelligence Quotient.

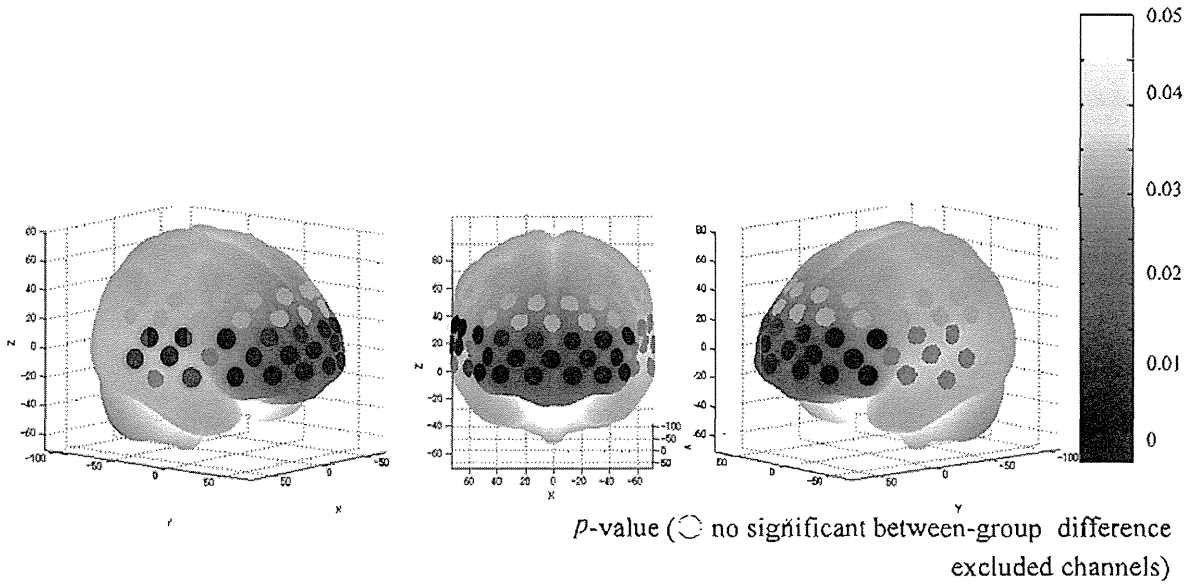

Fig. 2. p-value significance map of t-tests for oxy-Hb increases in patients with MDD compared with healthy controls during VFT using FDR correction. The warm colored circles represent significantly smaller oxy-Hb increases than in the control group at the channels indicated. There were 22 channels (ch22–29, ch32–33, ch35–39 and ch44–50; FDR-corrected p: 000–.024).

to −.48; FDR-corrected p: .002 to .007). Mean oxy-Hb changes showed no significant correlations with doses of anxiolytics.

6. Discussion

6.1. Task performance

The number of words generated during the VFT did not differ significantly between patients and controls, which is consistent with the majority of previous studies (Matsuo et al., 2002; Fossati et al., 2003; Suto et al., 2004; Kameyama et al., 2006). Previous studies reported impairment on semantic fluency tasks in depression (Calev et al., 1989; Tarbuck and Paykel, 1995). However, on phonemic fluency task conflicting results patients showing normal or impairment performance in depression (Albus et al., 1996; Degl'Innocenti et al., 1998). Type of psychiatric disorder and task time setting may reflect the discrepancies (Fossati et al., 2003). In the present study, the time setting of VFT was three phonemes within 60 s, that is, 20 s for each phoneme, which differs from the standard VFT usually using 60 s for one phoneme. The time setting condition was designed as it is, so that the subjects were able to keep generating words regularly within the task period to avoid the effect of "not speaking". It is possible that the time setting condition in the present study caused the lack of significant between group-difference in task performance.

6.2. Between-group comparison of oxy-Hb activation

The present study showed oxy-Hb activation during VFT to be significantly smaller in the MDD group than in age-, gender- and IQ-matched healthy controls. This result is essentially consistent with those obtained using NIRS (Matsuo et al., 2002; Herrmann et al., 2004; Suto et al., 2004; Kameyama et al., 2006; Pu et al., 2008), single photon emission computed tomography (SPECT) (Mayberg et al., 1994) or functional magnetic resonance imaging (fMRI) (Okada et al., 2003).

6.3. Relationships with symptom severity at the time of examination

Mean oxy-Hb changes during the task period showed a significantly negative correlation with HAM-D21 total score at ch25. Ch25 is located approximately in the right DLPFC. The finding is in line with some initial studies (Bench et al., 1995; Mayberg et al., 1994) which suggest that abnormal functions in DLPFC are mood dependent. However, other more recent studies investigating cross-sectional relationship between depression psychopathology and brain function do not coincide with our result (Périco et al., 2005; Milak et al., 2005). One of the reasons for the discrepancy may arise from the different methodologies; in the present study we adopted VFT for activation whereas the previous studies observed the basal activity with no activation task. Although speculative as it is, the activation of PFC by VFT may have led to the significant relationship between oxy-Hb changes and depression symptom severity in the right DLPFC.

More interestingly, mean oxy-Hb changes during the task period showed significant negative correlations with three individual HAM-D21 items in a wider area than they showed with HAM-D21 total scores; insomnia early in nine, work and activity in two and psychomotor retardation in twelve channels. The nine channels correlating with "insomnia early" were located approximately in the right pre-motor area, DLPFC and frontopolar and orbitofrontal areas. The two channels correlating with "work and activity" were located approximately in the right DLPFC and temporopolar area. The twelve channels correlating with "psychomotor retardation" were located broadly in the fronto-temporal areas with right hemispheric dominance. Although these findings should be treated with care given the exploratory nature of multiple analyses, it is noteworthy that at least some subscale scores of HAM-D21 appeared to show stronger relationship with oxy-Hb changes than HAM-D21 total scores. It has been pointed out that HAM-D17 and/or HAM-D21 are not necessarily unidimensional, and thus not adequate to assess depression severity (Bagby et al., 2004). Licht et al. (2005) showed that a set of the HAM-D containing six subscales constitute a unidimensional scale measuring severity of

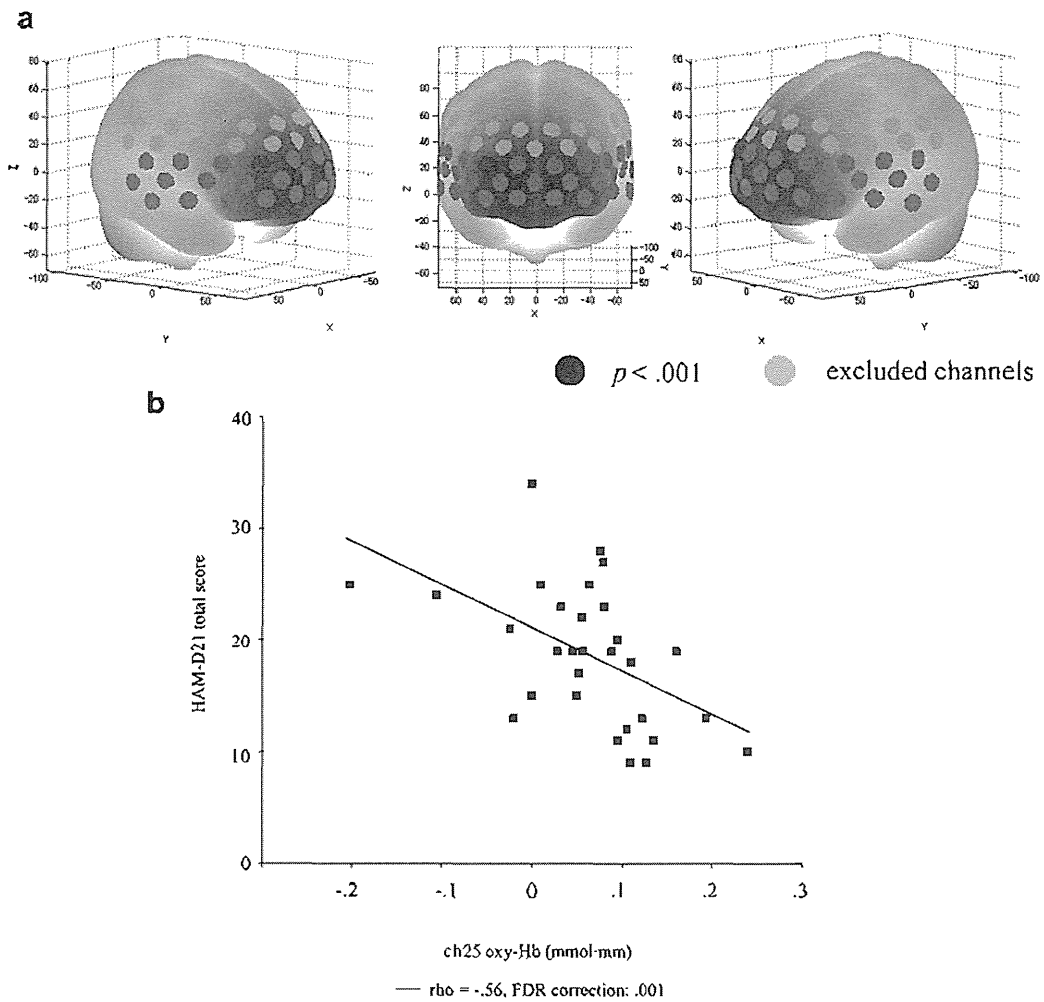

Fig. 3. (a) The channels with a significant correlation between oxy-Hb changes and HAM-D21 total score after FDR correction. (b) Scatter graph showing the relationship between HAM-D21 total scores and oxy-Hb activation in ch25.

depression, whereas the remaining items covering neurovegetative symptoms showed a problematic response somewhat insensitive to depression severity. In fact, the multidimensionality was highlighted in the unstable factor structure, which was demonstrated by a failure to replicate a single unifying structure across studies (Bagby et al., 2004). The relatively strong relationship indicated between HAM-D21 subscale scores and oxy-Hb changes in divergent areas, compared to HAM-D21 total scores may be due to the multidimensional properties of HAM-D21. Graff-Guerrero et al. (2004) also demonstrated that each HAM-D subscale score showed a significant correlation with the basal CBF in variant areas, in some cases showing positive correlation and others negative.

6.4. Relationships with medications

As all patients were taking antidepressants at the time of evaluation, the medication effect could not be ignored. Yet, there was no significant relationship between daily dose levels of antidepressants and the HAM-D21 total score. Although daily dose levels of antidepressants showed significant negative correlations with oxy-Hb changes in six channels, ch25, where a significant correlation between oxy-Hb changes and HAM-D21 total scores was observed, was not included in the six channels. Therefore, we suspect that the effect was small, if at all.

PET has been used to demonstrate that antidepressant medication normalizes both over-activity and under-activity in the frontal cortex (Kennedy et al., 2001, 2007; Mayberg et al., 2000; Goldapple et al., 2004). Unfortunately, our results could not clarify the relationship between medication and brain activation because our analysis was based on cross-sectional data. Although our data may reflect the more restraint-free, natural setting than those using fMRI or PET, further studies in drug-naïve patients are required to draw any conclusions as to the possible effects of medication on brain activation as measured by NIRS. Longitudinal studies investigating the relationship between the change in oxy-Hb data and symptom severity scores with a larger sample size are warranted to reach a conclusion on this matter.

The results of this study must be interpreted with caution due to certain limitations. First, because the analysis was based on cross-sectional data, causality cannot be determined. Longitudinal studies are needed to assess cause-and-effect relationships. Second, our sample size was not large, and is thus subject to type II error. Further studies with larger numbers of MDD patients are required. Finally, owing to the multidimensional properties of HAM-D21, assessment of depression symptom severity using HAM-D21 total scores may not be adequate, and thus, other scales such as Montgomery Asberg Depression Rating Scale (MADRS) or Beck Depression Inventory (BDI) should be tested in the future study.

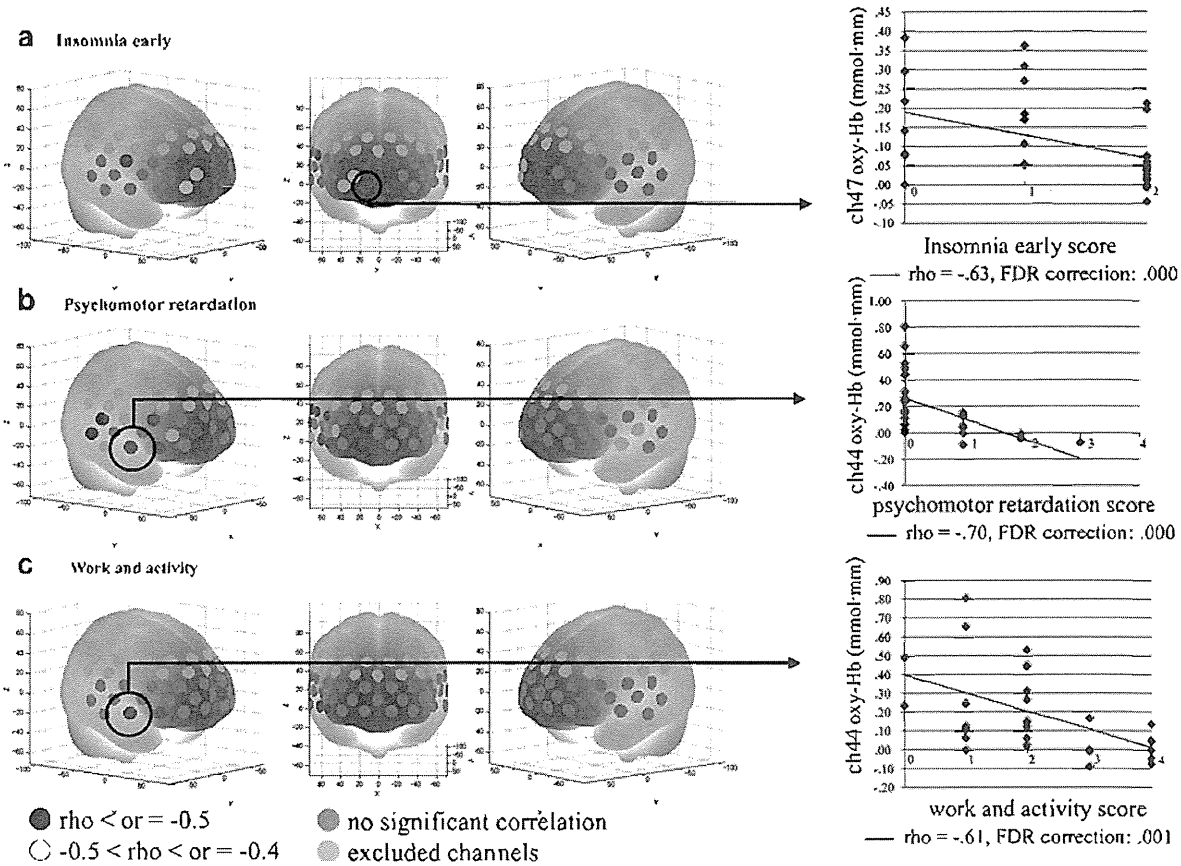

Fig. 4. rho-value map for the correlation between oxy-Hb activation in MDD patients and three individual HAM-D21 subscale scores after FDR correction. (a) insomnia early, (b) psychomotor retardation, and (c) work and activity.

7. Conclusion

In this study, we confirmed that the increase in oxy-Hb during a VFT task is significantly smaller in MDD than in age- and gender-matched healthy subjects. This difference could not be explained by a difference in task performance or premorbid IQ. The blunted increase in right DLPFC was associated with the symptom severity of MDD and therefore oxy-Hb changes during VFT in this region may be a state-dependent marker of depression.

Role of the funding source

This study was supported by an Intramural Research Grant (20-3; 21-9) for Neurological and Psychiatric Disorders of NCNP, and Health and Labor Sciences Research Grants (Comprehensive Research on Disability, Health and Welfare) and research grants from the Japanese Ministry of Health, Labour and Welfare (H22-seishin-ippan-001 and Comprehensive Research on Disability Health and Walfare).

Contributors

T. Noda designed the study, wrote the protocol, assessment of depression severity, literature searches, statistically analyzed the data, and wrote the first draft of the manuscript. T. Matsuda was involved in patient recruitment and assessment of depression severity. H. Kunugi and S. Yoshida wrote the final version of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All the authors declare that they have no conflicts of interest with respect to this study or its publication.

Acknowledgments

The authors thank all the participants in this study. We thank Mr. Yuji Sugimura and Mr. Masaru Ogawa, who support NIRS measurement. We are also grateful to Dr Kazuyuki Nakagome for helpful suggestions and observations and a critical reading of the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpsychires.2012.04.001.

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