平成28年度~平成30年度

森林浴による健康増進等に関する調査研究

報告書

公益財団法人 車両競技公益資金記念財団

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はじめに

世界的に温暖化や経済活動により多くの緑が消えていく中、我が国は豊かな森林資源を 持ち、1982年に林野庁によって提唱された森林浴はその後の健康志向とも相まって広く社 会に広がりました。

しかしながら、その効用については医学的データが少なく、客観的な根拠が乏しい状況と なっておりました。近年、データ蓄積によって森林セラピーが生み出す生理的効果は明らか となってきていますが、これらデータの多くは 20 代の健康な男性において取得されたもの であり、最も森林セラピーが必要と考えられる人々のデータ蓄積は皆無に近いのが現状で あります。

そこで、私ども車両競技公益資金記念財団は森林浴による健康増進等に関する調査研究 事業委員会を設置し、平成25年度からの第I期3年間でデータ蓄積が少ない人々を被験者 として森林セラピーの生理的、心理的リラックス効果についてデータ収集を進め、その効果 について多角的に検討するとともに、平成28年度からの第II期3年間で視覚、嗅覚、聴覚 刺激に分けて、それらの単独刺激ならびに複合刺激実験を実施し、森林浴効果のメカニズム の解明を行い、この度、第II期研究成果を報告書として取りまとめることといたしました。 この研究成果について、より広く社会の各方面に公表することが必要との声もあり、刊行す る次第です。

健康増進に関する諸研究の一助となれば幸いです。

令和2年1月

公益財団法人車両競技公益資金記念財団

理事長 深澤 亘

平成28年度

森林浴による健康増進等に関する調査研究

報告書

千葉大学環境健康フィールド科学センター

宮崎良文

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はじめに

森林セラピー研究は、日本において1990年代に始まり、ここ十数年で多くの生理デ ータが蓄積されてきた。森林セラピー実験には、森林にて実施する「フィールド実 験」と人工気候室内にて実施する「室内実験」が存在する。

これまでの森林セラピー研究においては、「フィールド実験」「室内実験」ともに 本研究課題実行者が中心となり、生理的リラックス効果に関するデータを提出してき た。これは世界に類を見ない科学的蓄積である。

「室内実験」においては、五感に関わる一刺激実験が実施され、その生理的メカニ ズムの解明に貢献してきた。しかし、これまで「室内実験」における自然由来の複合 刺激がもたらす生理的効果に関するデータは、報告されていない。

そこで、今年度は、1)大型ディスプレイを用いた森林視覚刺激実験、2)視覚なら びに嗅覚複合刺激実験を行った。さらに、3)脊髄損傷車椅子患者に対する森林盆栽視 覚刺激実験を実施した。

I 大型ディスプレイ視覚刺激が前頭前野活動に及ぼす影響

(1) はじめに

森林セラピー研究においては、心拍変動性、唾液中コルチゾール濃度等の各種の生理 指標を用いたフィールド実験が実施され、森林環境がもたらす生理的リラックス効果が 明らかになりつつある。

しかし、脳活動に及ぼす影響に関しては、フィールド実験ならびに室内実験共に、デ ータ蓄積は極めて少ないのが現状である。

本研究においては、人工気候室にて大型ディスプレイによる森林画像を用いることに より、森林の視覚刺激が前頭前野活動に及ぼす経時的影響を明らかにすることを目的と した。

(2) 方法

材料と方法の概要を図1に示す。

- 実験場所:千葉大学環境健康フィールド科学センター
 人工気候室(室温24℃、湿度50%)
- ●被験者:女子大学生17名(21.1±1.0歳)
- ●刺激:90秒間(森林画像:メタセコイア林、都市画像:新宿)
- ●測定項目:生理指標:近赤外時間分解分光法(TRS)
 ⇒前頭前野酸素化ヘモグロビン濃度 (TRS-20、浜松ホトニクス(株))

主観評価:簡易SD法

☆「快適感」「リラックス感」「自然感」

●検定:生理指標 ⇔ 対応のあるt検定(片側)+Holm補正
 主観評価 ⇔ ウィルコクソンの符号付順位和検定(片側)

図1 材料と方法の概要

実験場所は、千葉大学環境健康フィールド科学センター内の人工気候室(室温 24℃、 湿度 50%、照度 2-4 lx)とし、被験者は女子大学生 17 名(21.1±1.0 歳)とした。

視覚刺激はパナソニック製 85 インチ (TH-85AX900)、画素数 3840×2160 dpi の大型ディスプレイ (図 2) を用いて、90 秒間 とした。森林画像としてはメタセコイア林を用い、都市画像としては新宿高層ビル群とした (図 3)。

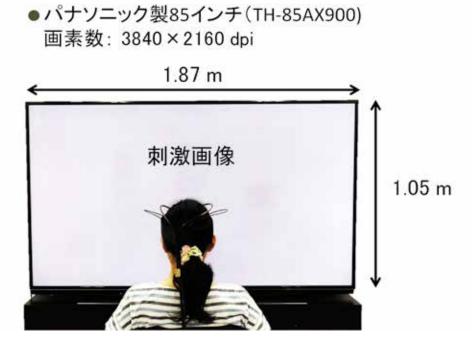


図2 ディスプレイ



森林画像

都市画像

図3 実験に用いた画像

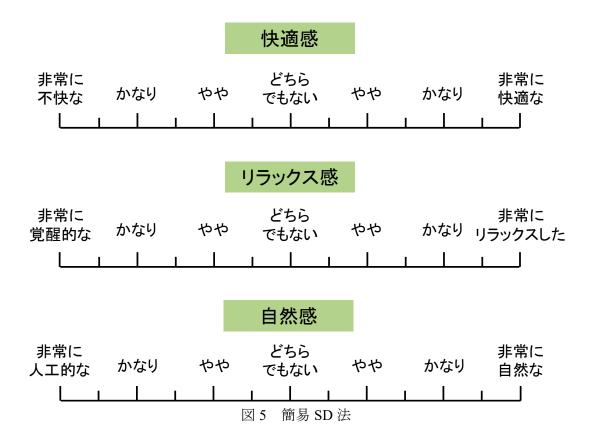
生理指標としては、近赤外時間分解分光法((TRS-20、浜松ホトニクス㈱))による近前 頭前野酸素化ヘモグロビン濃度を用いた(図4)。



TRS(近赤外時間分解分光法; Time-Resolved Spectroscopy)

図4 TRS による酸素化ヘモグロビン濃度計測の風景

主観評価としては、簡易 SD 法を用いて「快適感」 「リラックス感」 「自然感」を 13 段階にて計測した(図 5)。



実験プロトコルを図6に示す。

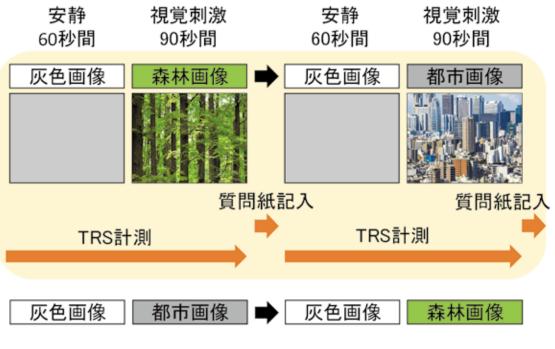


図6 実験プロトコル

安静時間は 60 秒間とし、森林あるいは都市視覚刺激(図 7)の 90 秒後に質問紙に記入し、主観評価を実施した。TRS は毎秒計測した。森林画像と都市画像の呈示順については、カウンターバランスを取って、刺激順の影響が出ないようにした。



森林画像

都市画像

図7 実験風景

検定は、生理指標においては、対応のある t 検定(片側)を用い、多重比較には Holm 補 正を行った。主観評価においては、ウィルコクソンの符号付順位和検定(片側)を用い た。

(3) 結果と考察

右前頭前野における酸素化ヘモグロビン濃度の1秒毎の経時的変化を図8に示す。 森林画像における酸素化ヘモグロビン濃度は、都市画像に比べ、低く推移している。

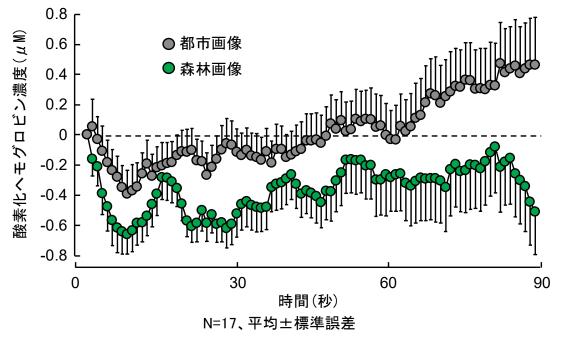
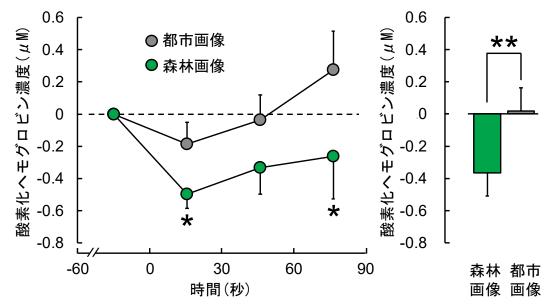


図8 右前頭前野における酸素化ヘモグロビン濃度の1秒毎の経時的変化

右前頭前野における酸素化ヘモグロビン濃度の 30 秒毎の経時的変化を図 9 左に示 す。1-30 秒および 61-90 秒の酸素化ヘモグロビン濃度は、森林画像において都市画像 に比べ、有意に低下した。

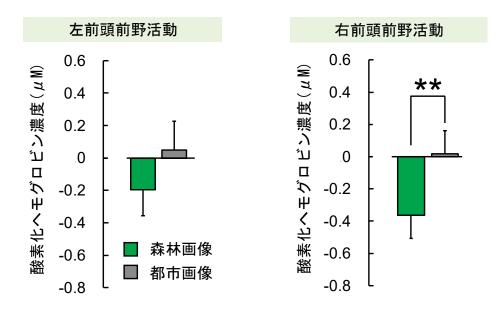


N=17、平均±標準誤差、*: p<0.05、**: p<0.01、対応のあるt検定(Holm補正)

図9 右前頭前野における酸素化ヘモグロビン濃度 図9右に90秒間の平均値を示す。

森林画像においては-0.36±0.14µM、都市画像では 0.02±0.14µM となり、森林画像において有意な低下を示した。

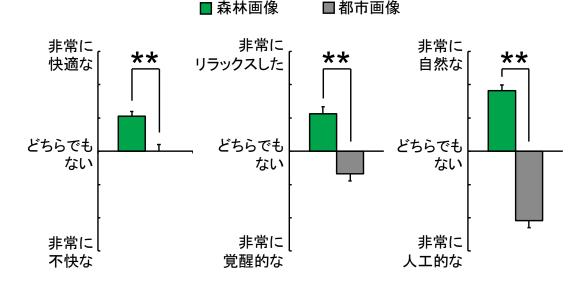
図10に左右前頭前野活動の平均値を示す。



N=17、平均±標準誤差、**: p<0.01、対応のあるt検定

図 10 左右前頭前野における酸素化ヘモグロビン濃度

簡易 SD 法においては、森林画像は「快適感」、「リラックス感」、「自然感」が有意 に高まることが分かった(図 11)。



N=17、平均±標準誤差、**: p<0.01、ウィルコクソンの符号付順位和検定

図 11 簡易 SD 法

結論として、森林の視覚刺激によって、(1)右前頭前野の酸素化ヘモグロビン濃度が 有意に低下し、右前頭前野活動が鎮静化すること、(2)「快適感」、「リラックス感」な らびに「自然感」が有意に高まることが明らかになった。

Ⅱ 視覚・嗅覚複合刺激が前頭前野活動・自律神経活動に及ぼす影響

(1) はじめに

森林セラピーがもたらす健康増進効果が明らかになりつつあり、そのメカニズムの解 明は重要な研究課題の一つになっている。これまで我々は、五感を介するフィールド実 験と共に、各感覚刺激による室内実験の両面からデータを蓄積してきた。室内実験にお いては、視覚ならびに嗅覚の単独刺激による検証は行われているが、複合刺激がもたら す影響に関しては検討されていない。

そこで、本研究においては、森林の視覚ならびに嗅覚の複合刺激がもたらす生理的影響を前頭前野活動と自律神経活動を指標として明らかにすることを目的とした。

(2) 方法

女子大学生 21 名(21.1±1.0 歳)を被験者とし、人工気候室(温度 24℃、湿度 50%、照度 50 lx)において実施した。被験者情報を図 12 に示す。

sub	身長	体重	年齢	視力矯正の有無	右	左
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	157	55	20	矯正(コンタクトレンズ)	1.0	1.0
4	158.9	52.1	20	矯正(メガネ)	0.5	0.5
5	158	58	21	矯正(メガネ)	1.0	1.0
6	162	51	20	矯正(メガネ)	1.0	1.0
7	162	54	21	矯正(コンタクトレンズ)	1.0	1.0
8	152.8	46.7	23	矯正(コンタクトレンズ)	1.0	1.0
9	154	55	21	矯正(コンタクトレンズ)	1.0	1.0
10	158	48	20	矯正(コンタクトレンズ)	0.8	0.8
11	155	48	21	矯正(?)	1.0	1.2
12	157	57	22	矯正(メガネ)	0.5	0.5
13	157	48	23	裸眼	1.0	1.0
14	-	-	-	-	-	-
15	153	56.1	23	矯正(コンタクトレンズ)	1.0	1.0
16	158.2	56	21	矯正(メガネ)	1.0	0.9
17	168	63	21	裸眼	1.0	1.0
18	157	60	22	裸眼	1.5以上	1.5以上
19	152	43	20	矯正(メガネ)	1.0	1.0
20	161	57	22	矯正(コンタクトレンズ)	1.0	1.0
21	167	50	21	裸眼	0.7	0.7
22	158	39	21	矯正(コンタクトレンズ)	1.0	1.0
23	164	51	21	裸眼	1.5以上	1.5以上
24	153	42	20	矯正(コンタクトレンズ)	1.0	0.8
25	-	-	-	-	-	-

	年齢	身長	体重
平均	158.2	51.9	21.1
標準偏差	4.5	6.2	1.0
標準誤差	1.0	1.3	0.2

図 12 被験者情報一覧

刺激一覧を図13に示す。

1視覚刺激:赤沢自然休養林風景画像
 2嗅覚刺激:ヒノキ葉油(2µℓ/24ℓ)
 3複合刺激:上記①+②
 ④対照:グレー画像+嗅覚刺激なし

図13 刺激一覧

視覚刺激としてヒノキ林の写真(図 14)を用い、パナソニック製 85V型のディスプ レイ(TH-85AX900;幅 1,872mm×高さ 1,053mm)により呈示した(図 15)。

視覚刺激:赤沢自然休養林風景画像



図 14 視覚刺激として用いた画像



図15 大型ディスプレイの概要

嗅覚刺激は、ヒノキ葉油(図 16) 2µℓ をにおい袋(24ℓ)に注入し、鼻下約 15cm から 3.0ℓ/分にて供給した(図 17)。



嗅覚刺激:ヒノキ葉油(2μθ)

図16 嗅覚刺激として用いたヒノキ葉油



図17 実験風景1

複合刺激は、視覚ならびに嗅覚刺激を同時に呈示することとし、コントロールは、刺激なしとした。刺激時間は 90 秒間とし、提示する刺激の順番はカウンターバランスを とった(図 18)。

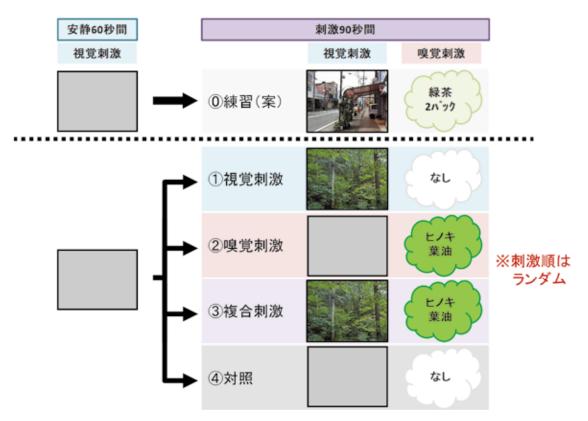


図18 実験プロトコル

実験風景を図 19, 20 に示す。



図 19 実験風景 2



図 20 実験風景 3

脳活動の指標は、近赤外時間分解分光法(TRS-20、浜松ホトニクス株式会社)による左 右前頭前野における酸素化ヘモグロビン濃度とした。自律神経活動の指標は、心拍変動 性ならびに心拍数とし、携帯型心電図モニター(Activtracer AC-301A、GMS)を用いてデ ータを取得した。ln(HF)を副交感神経活動の指標とし、ln(LF/HF)を交感神経活動の指標 として用いた。主観評価は、簡易 SD 法とし、「快適感」、「リラックス感」、「自然感」、 「臨場感」について 13 段階で評価した。

統計検定は、生理指標においては対応のある t 検定(片側検定)を行い、主観評価においてはウィルコクソンの符号付順位和検定(片側検定)を実施した。有意水準は p<0.05 とし、多重比較には、Holm 補正を用いた。

(3) 結果と考察



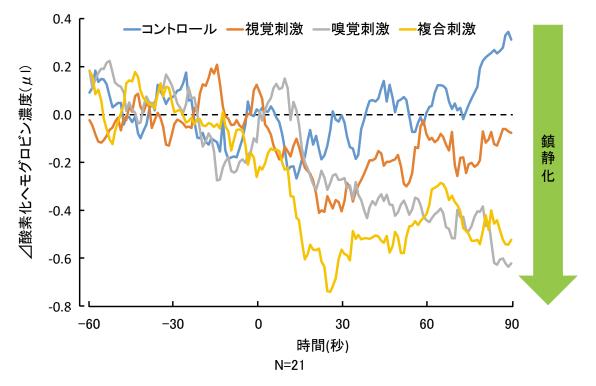
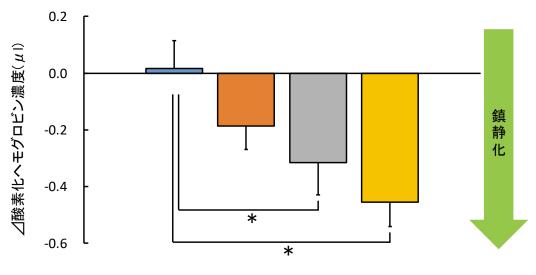


図 21 右前頭前野における酸素化ヘモグロビン濃度の変化

図 22 に示すように、森林の嗅覚刺激ならびに複合刺激時の酸素化ヘモグロビン濃度 は、コントロールに比べ、有意に低下した。左前頭前野においても同様の傾向が見られ、 複合刺激とコントロールの間に有意差が認められた。

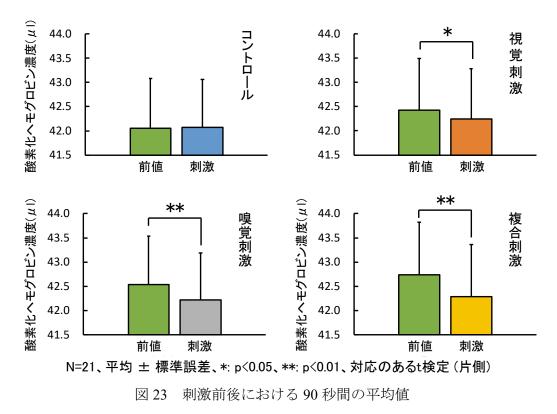


コントロール 視覚刺激 嗅覚刺激 複合刺激

N=21、平均 ± 標準誤差、*: p<0.05、対応のあるt検定 (片側・Holm補正)

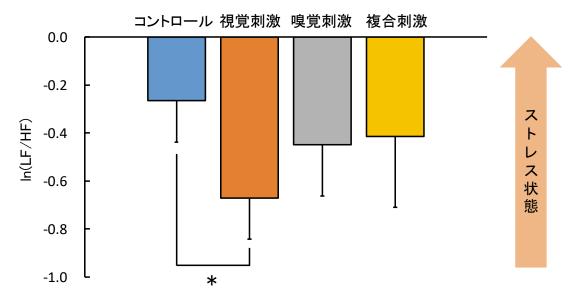
図 22 右前頭前野における酸素化ヘモグロビン濃度の 90 秒間の平均値

刺激前後における 90 秒間の平均値については、図 23 に示す。



交感神経活動の指標である ln(LF/HF)の結果を図 24 に示す。

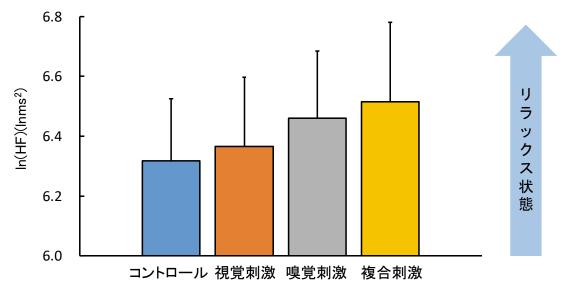
視覚刺激においてコントロールに比べ、有意に低下することがわかった。嗅覚ならび に複合刺激においても低下したが、有意差は認められなかった。



N=17、平均 ± 標準誤差、*: p<0.05、対応のあるt検定(片側・Holm補正)

図 24 交感神経活動における 90 秒間の平均値

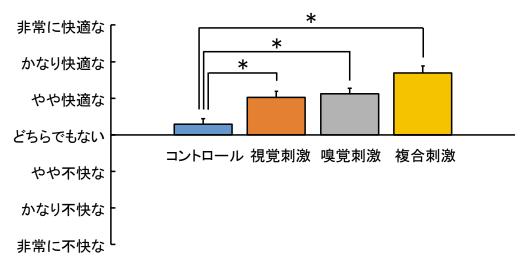
副交感神経活動の指標である ln(HF) (図 25) と心拍数において有意差はなかった。



N=17、平均 ± 標準誤差、対応のあるt検定(片側・Holm補正)

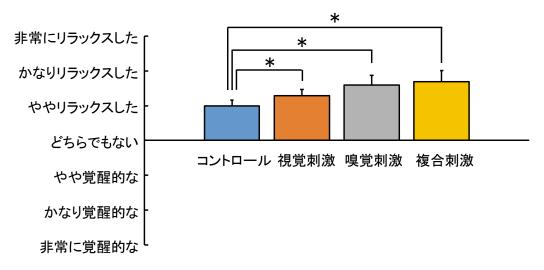
図 25 副交感神経活動における 90 秒間の平均値

簡易 SD 法においては、図 26~29 に示すように、視覚刺激、嗅覚刺激ならびに複合 刺激によって、「快適感」と「リラックス感」が有意に高まり、視覚刺激と複合刺激に よって、「自然感」と「臨場感」が有意に高まることがわかった。



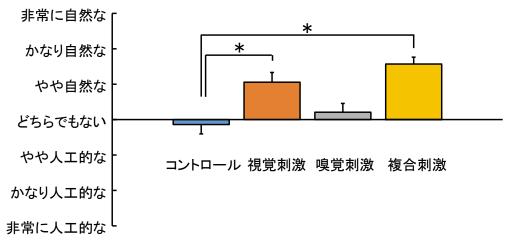
N=21、平均 ± 標準誤差、 *: p<0.05、ウィルコクソンの符号付順位和検定(片側・Holm補正)

図 26 簡易 SD 法における「快適感」

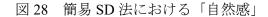


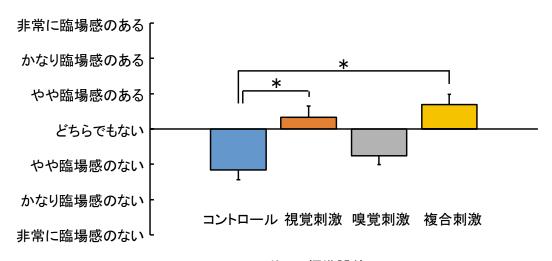
N=21、平均 ± 標準誤差、 *: p<0.05、ウィルコクソンの符号付順位和検定(片側・Holm補正)

図 27 簡易 SD 法における「リラックス感」



N=21、平均 ± 標準誤差、 *: p<0.05、ウィルコクソンの符号付順位和検定(片側・Holm補正)





N=21、平均 ± 標準誤差、 *: p<0.05、ウィルコクソンの符号付順位和検定(片側・Holm補正)

以上より、森林の視覚・嗅覚の単独および複合刺激は、生理的・心理的リラックス効 果をもたらすことが明らかになった。

図 29 簡易 SD 法における「臨場感」

Ⅲ ヒノキ林盆栽による視覚刺激が脊髄損傷患者車椅子利用者の前頭前野活動・自律神 経活動に及ぼす影響

(1) はじめに

近年、森林等の自然環境がもたらす生理的リラックス効果に関心が集まり、科学的デ ータの蓄積が進みつつある。一方、身体障碍者等においては、森林等に赴くことが難し いため、生活に取り入れられる「自然」への期待が高まっている。

日本において、盆栽は、古くから日常生活の中に取り入れられてきた「自然」のひと つである。盆栽は、自然景観を模して造形する点に特徴があり、最近では、代表的な日 本文化として、海外でも注目を集めている。しかし、盆栽の視覚刺激が生理応答に与え る影響を調査した研究は存在しない。

そこで本研究では、盆栽の視覚刺激が脊髄損傷者の前頭前野活動および自律神経活動 にもたらす影響を明らかにすることを目的とした。

(2) 方法

本実験は、千葉大学環境健康フィールド科学センター内の会議室にて行った。被験者 は、車椅子にて自走可能な男性脊髄損傷者 24 名(平均±標準偏差:49.0±16.4 歳)とした。 被験者情報を図 30 に示す。

sub	年齡	身長	体重
1	44	174	70
2	43	172	64
3	48	170	80
4	79	168	68
5	45	173	65
6	25	168	60
7	29	165	68
8	25	178	67
9	44	168	56
10	35	175	72
11	42	182	73
12	59	162	65
13	66	165	52
14	68	173	60
15	63	178	75
16	76	164	55
17	64	162	57
18	42	175	70
19	25	174	59
20	46	170	70
21	64	167	70
22	68	175	65
23	39	178	94
24	-	-	-
25	37	178	78

	年齡	身長	体重
平均	49.0	171.4	67.2
標準偏差	16.4	5.6	9.2
標準誤差	3.3	1.1	1.9

く募集要項>

成人男性(脊髄損傷者)

・車椅子を自力で操作でき、座位を保持できる方
 ・重篤な基礎疾患(糖尿病等)を有しない方

図 30 被験者情報一覧

視覚刺激は、図 31 に示すように、樹齢 10 年のヒノキ 8 本を用いた寄せ植え盆栽とした。対照は、盆栽なしとした。なお、盆栽および対照は、視覚刺激開始まで段ボールにて覆われていた。



図 31 刺激に用いた盆栽

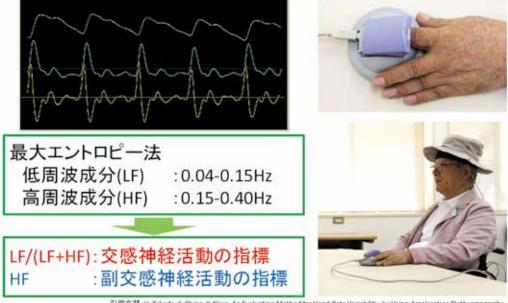
脳活動の指標は、近赤外分光法(Pocket NIRS、(株)ダイナセンス)による左右前頭前野酸素化ヘモグロビン濃度とした(図 32)。



図 32 携帯型近赤外線組織酸素モニター装置

自律神経活動の指標は、加速度脈波測定システム(アルテット、(株)ユメディカ)を用い、指尖加速度脈波による心拍変動性を測定した(図 33)。

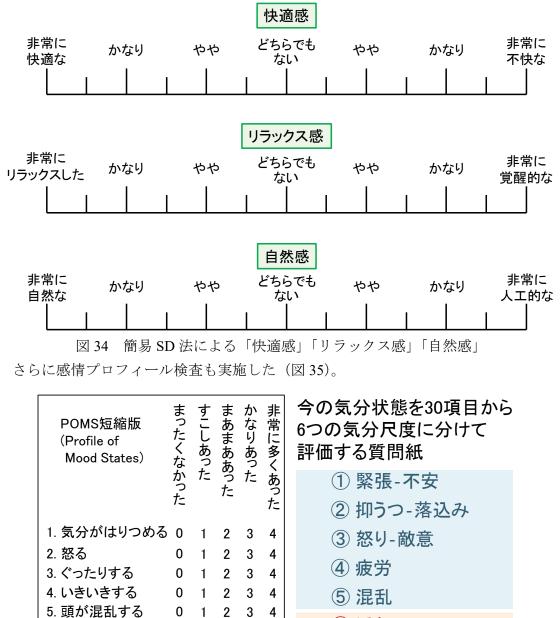
周波数解析は最大エントロピー法を用いた。HFを副交感神経活動の指標とし、LF/HF を交感神経活動の指標とし、正規化するために、自然対数変換値を用いた。



引用文献: H. Takada, K. Okino, Y. Niwa. An Evaluation Method for Heart Rate Variability, by Using Acceleration Plethysmography Health Evaluation and Promotion, 547-551, Vol. 31, No. 4, 2004.

図 33 指尖加速度脈波による心拍変動性(HRV)

主観評価としては、簡易 SD 法を用いて「快適感」 「リラックス感」 「自然感」を 13 段階にて計測した(図 34)。



⑥ 活気

図 35 気分プロフィール検査(Profile of Mood States, POMS) 短縮版

4

図 36、37 に実験プロトコルを示す。

0 1 2 3 4

30.活気がわいてくる 0 1 2 3

6. 落ち着かない

被験者は、前室にて実験の説明を受け、同意書へ署名し、会議室に移動した。生理計 測用のセンサーを装着した後、開眼にて約1分間安静状態を保った。その後、実験者が 段ボールを外し、盆栽あるいは対照の視覚刺激を1分間受けた。なお、刺激の呈示順は、 カウンターバランスをとった。

車内(控え室) (担当:宮崎•落合)					内(実験室) :宋・池井)		
25分		2分	2分	8分	8分	8分	1分
実験の説明 同意書 パーソナリティテスト 主観評価練習	移動	NIRS 装着	流れ 説明	練習刺激	視覚刺激 盆栽 あり or なし	視覚刺激 盆栽 なし or あり	NIRS 取り外し

and the second se						
			8分			
60秒	60秒	30秒	60秒	2分	30秒	2分
教示	安静	箱 取外し	視覚刺激	主観 評価	Sub 後ろ 向く	視覚 刺激 入替

※刺激順はカウンターバランスをとった

図 36 実験プロトコル (詳細)

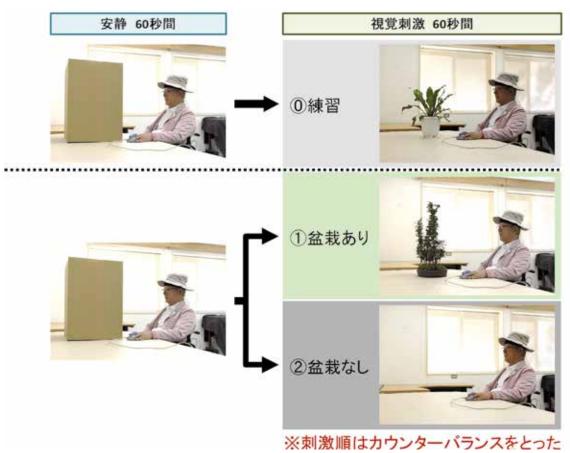


図 37 実験プロトコル

さらに、実験風景を図38に示す。



図 38 実験風景

統計検定は、生理指標においては対応のある t 検定(片側)を実施した。有意水準は p<0.05 とした。

(3) 結果と考察

盆栽視覚刺激時の左前頭前野酸素化ヘモグロビン濃度を図 39 に示す。

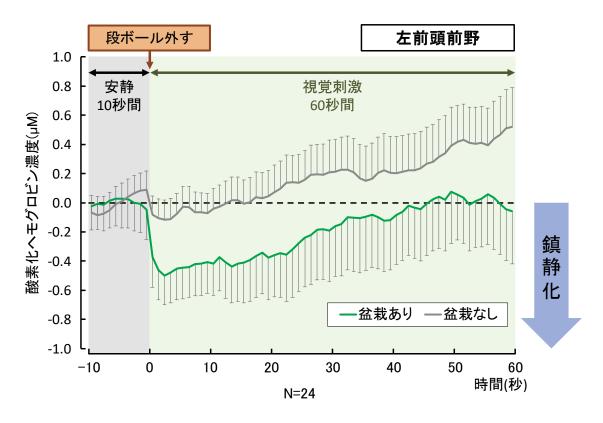
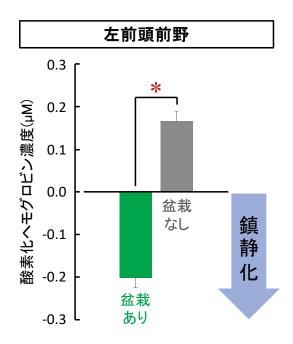


図 39 左前頭前野における酸素化ヘモグロビン濃度の1秒毎の経時的変化

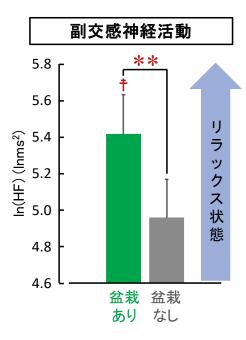
盆栽視覚によって、対照と比較し、有意に低下することが明らかとなった(盆栽:-0.20±0.02μM、対照:0.17±0.02μM、p<0.05、図40)。一方、右前頭前野においては、有意な差はなかった。



N=24、平均 ± 標準誤差、*p<0.05、対応のあるt検定(片側)

図 40 左前頭前野における酸素化ヘモグロビン濃度の 60 秒間の平均値

副交感神経活動の指標である ln(HF)の結果を図 41 に示す。

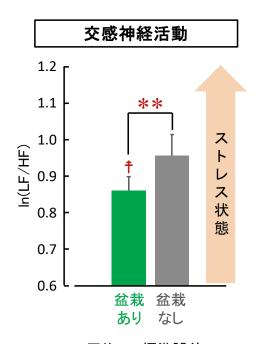


N=24、平均 ± 標準誤差、 **p<0.01: 盆栽ありなしの比較(対応のあるt検定・片側) †p<0.05: 盆栽視覚刺激前後の比較(対応のあるt検定・片側)

図 41 副交感神経活動における 60 秒間の平均値

盆栽の視覚刺激によって、対照と比べ、有意に上昇した(盆栽: 5.45±0.23lnms²、対照: 4.95±0.21lnms²、p<0.01)。

図 42 に交感神経活動の指標である ln(LF/HF)の結果を示す。盆栽の視覚刺激によって、対照と比較し、有意な低下が示された(盆栽:0.85±0.04、対照:0.95±0.06、p<0.05)。



N=24、平均 ± 標準誤差、 **p<0.01: 盆栽ありなしの比較(対応のあるt検定・片側) †p<0.05: 盆栽視覚刺激前後の比較(対応のあるt検定・片側)

図 42 交感神経活動における 60 秒間の平均値

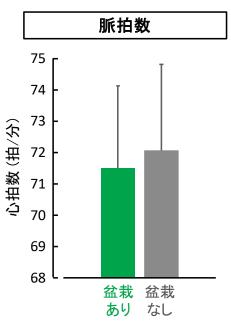
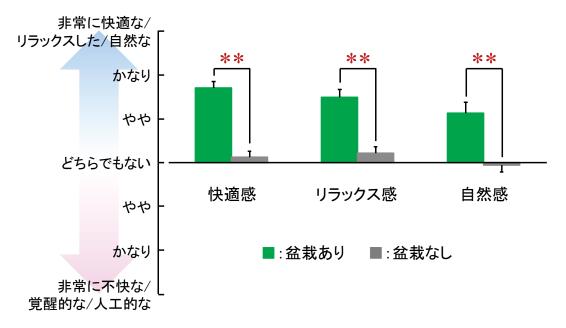


図43に示すように、脈拍数には変化がなかった。

N=24、平均 ± 標準誤差

図 44 に簡易 SD 法の結果を示す。盆栽の視覚刺激によって、「快適感」「リラックス 感」「自然感」が有意に高まることが明らかとなった。

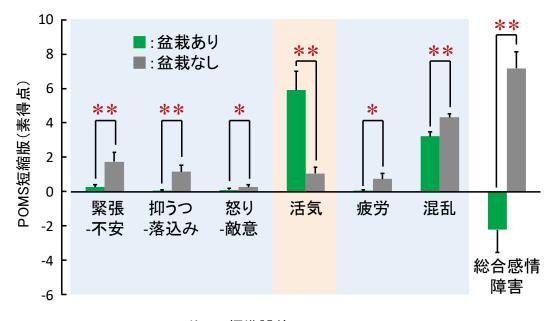


N=24、平均 ± 標準誤差、**p<0.01、ウィルコクソンの符号付順位和検定(片側)

図 44 簡易 SD 法による「快適感」「リラックス感」「自然感」

図 43 脈拍数における 60 秒間の平均値

図 45 に POMS 短縮版の結果を示す。



N=19、平均 ± 標準誤差、*p<0.05、**p<0.01、 ウィルコクソンの符号付順位和検定(片側)

図 45 POMS 短縮版

盆栽の視覚刺激によって、「緊張-不安」「抑うつ-落ち込み」「怒り-敵意」「疲労」 「混乱」の各感情尺度得点と「総合感情障害」得点が低下し、「活気」尺度が上昇する ことがわかった。

結論として、盆栽の視覚刺激は、脊髄損傷者に対して、生理的リラックス効果ならび に心理的リラックス効果をもたらすことが明らかになった。

おわりに

今年度は、(1)大型ディスプレイを用いた森林視覚刺激実験、(2)視覚ならびに嗅 覚複合刺激実験を行った。さらに、(3)脊髄損傷車椅子患者に対する森林盆栽視覚刺 激実験を実施し、以下の結論を得た。

(1) 大型ディスプレイを用いた森林視覚刺激実験

森林の視覚刺激によって、1)右前頭前野の酸素化ヘモグロビン濃度が有意に低下し、右前頭前野活動が鎮静化すること、2)「快適感」、「リラックス感」ならびに「自然感」が有意に高まることが明らかになった。

(2) 視覚ならびに嗅覚複合刺激実験

本実験において、1)森林の嗅覚刺激ならびに複合刺激時の左右前頭前野における酸素化ヘモグロビン濃度は、有意に低下し、左右前頭前野活動が鎮静化すること、 2)視覚刺激において、交感神経活動が有意に低下し、ストレス状態が抑制される こと、3)すべての刺激において「快適感」と「リラックス感」が高まることがわかった。

(3) 脊髄損傷車椅子患者に対する森林盆栽視覚刺激実験

盆栽の視覚刺激によって、1)左前頭前野における酸素化ヘモグロビン濃度が有 意に低下し、左前頭前野活動が鎮静化すること、2)副交感神経活動が高まり、交 感神経活動が低下すること、3)「快適感」「リラックス感」「自然感」が高まり、POMS におけるすべての負の感情尺度が抑制され、「活気」が高まることが認められた。

以上より、上記の自然由来の刺激は生理的・心理的リラックス効果をもたらすこ とが明かとなった。今回の実験データはすべて世界初の知見である。

本研究は以下のメンバーの協力の元に実施された(五十音順)。

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- 宋チョロン(千葉大学環境健康フィールド科学センター)

平成29年度

森林浴による健康増進等に関する調査研究

報告書

千葉大学環境健康フィールド科学センター

宮崎良文

平成 29 年度目次

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はじめに

森林セラピー研究は、日本において1990年代に始まり、ここ十数年で多くの生理デ ータが蓄積されてきた。森林セラピー実験には、人工気候室内等にて実施する「室内 実験」と森林等で実施する「フィールド実験」が存在する。

これまでの森林セラピー研究においては、「室内実験」「フィールド実験」ともに 本研究課題実行者が中心となり、生理的リラックス効果に関するデータの多くを提出 してきた。これは世界に類を見ない科学的蓄積である。

「室内実験」においては、五感に関わる一感覚刺激実験が実施され、その生理的メ カニズムの解明に貢献してきた。一方、これまで「室内実験」における自然由来の複 合刺激がもたらす生理的効果に関しては、高い関心が持たれていたが、その報告例は なかった。昨年度の本調査研究において実施された「視覚刺激」と「嗅覚刺激」の複 合刺激実験が初の生理実験となる。

また、「フィールド実験」においては、近年の都市化に伴ったストレス状態を反映 して、自然由来の刺激が、健常者ではなく精神疾患患者にもたらす生理的影響に世界 の関心が集まっている。

そこで、今年度は、1)「室内実験」においては、大型ディスプレイを用いた「視覚 刺激」と森林ハイレゾ音を用いた「聴覚刺激」の複合刺激がもたらす生理的影響を明 らかにすること、2)「フィールド実験」においては、心療内科通院中のうつ病患者を 被験者として、病院外壁のビオトープがもたらす生理的影響を明かにすることを目的 とした。

(1)視覚および聴覚刺激がもたらす森林浴効果の解明-室内実験から-

(1) はじめに

森林セラピーがもたらす健康増進効果が明らかになりつつあり、そのメカニズムの解 明は重要な研究課題の一つになっている。これまで我々は、五感を介するフィールド実 験と共に、各感覚刺激による室内実験の両面からデータを蓄積してきた。

そこで、今年度は、人工気候室を使った室内実験において、視覚ならびに嗅覚の複合 刺激がもたらす影響を前頭前野活動と自律神経活動を指標として生理的に明らかにす ることを目的とした。

(2) 方法

皮験者番号	年齡	身長	体重	備考		年齡	身長	
sub01	21	159	50		平均	22.1	159.3	
sub02	21	160	51		標準偏差	1.8	4.1	
sub03	22	154	45		標準誤差	0.4	0.9	
sub04	28	159	46					
sub05	21	162	48					
sub06	23	163	60					
sub07	22	163	58					
sub08	21	152	41					
sub09	23	155	48					
sub10	-	—	-	×欠席のため、欠番				
sub11	20	158	46					
sub12	20	165	55					
sub13	22	158	44					
sub14	_	_	_	×欠席のため、欠番				
sub15	23	159	48					
sub16	21	167	55					
sub17	24	166	52					
sub18	22	158	45					
sub19	_	_	_	×欠席のため、欠番				
sub20	22	158	48					
sub21	_	_	_	×欠席のため、欠番				
sub22	24	152.8	46.7					
sub23	-	-	-	×欠席のため、欠番				
sub24	20	160	49					
sub25	22	157	55					

女子大学生 20 名(平均±標準偏差, 21.1±1.8 歳)を被験者とし、人工気候室(温度 25℃、 湿度 50%、照度 10 lx)において実施した。被験者情報を図1に示す。

図1 被験者情報一覧

図2に生理指標一覧を示す。自律神経活動は交感神経活動、副交感神経活動、心拍を 指標とし、主観評価は簡易型SD法、室内温冷感を指標とした。

】測定指標

(1)生理指標

1)自律神経活動 ①HRV(HF、LF/HF)		
②心拍数	 	連続測定
2)中枢神経活動		
③携帯型NIRSによる前頭前野酸素化ヘモグロビン濃度		
(2)主観評価	J	

1)簡易型SD法

「快適感」、「リラックス感」、「自然感」、「臨場感」

「音の感覚強度」

2) 部屋の「温冷感」の評価

図2 測定指標

視覚刺激は、図 3 に示すパナソニック製 85V型のディスプレイ(TH-85AX900; 幅 1,872mm×高さ 1,053mm)により呈示した。



図3 大型ディスプレイの概要

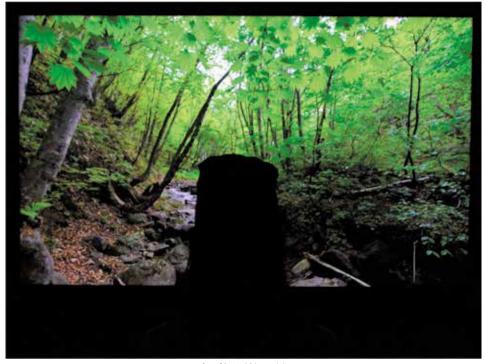


図4 視覚刺激風景 図4に視覚刺激時の実験風景を示す。照度は10lxとした。

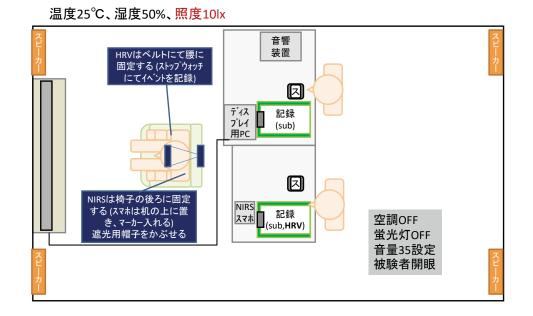


図5 人工機構室内のスピーカーの配置図

図 5 に人工気候室内におけるスピーカーの配置図を示す。音源は、「戸隠(11分15秒~12分45秒,90秒間)」とした。音量は、35(株式会社 JVC ケンウッド製4chアンプ,AX-D01SSD)に設定し、居室内に設置した4台のスピーカー(株式会社JVCケンウッド製スピーカー,SP-DD01SSD)から提示した。



図6 測定風景(左)ならびに前頭前野活動(右上)、自律神経活動(右下)計測風景

図6に測定風景を示す。脳活動の指標は、携帯型近赤外分光法(Pocket NIRS、(株)ダイ ナセンス)による左右前頭前野における酸素化ヘモグロビン濃度とした。自律神経活動 の指標は、心拍変動性ならびに心拍数とし、携帯型心電図モニター(Activtracer AC-301A、 GMS)を用いてデータを取得した。HF を副交感神経活動の指標とし、LF/HF を交感神経 活動の指標として用いた。主観評価は、簡易 SD 法とし、「快適感」、「リラックス感」、 「自然感」、「臨場感」について13 段階で評価した。

統計検定は、生理指標においては対応のあるt検定(片側検定)を行い、主観評価においてはウィルコクソンの符号付順位和検定(片側検定)を実施した。有意水準はp<0.05とし、多重比較には、Holm補正を用いた。

図7に刺激順を示す。複合刺激は、視覚ならびに聴覚刺激を同時に呈示することとし、対照は、刺激なしとした。刺激時間は90秒間とし、提示する刺激の順番はカウンターバランスをとった。図8に実験風景を示す。

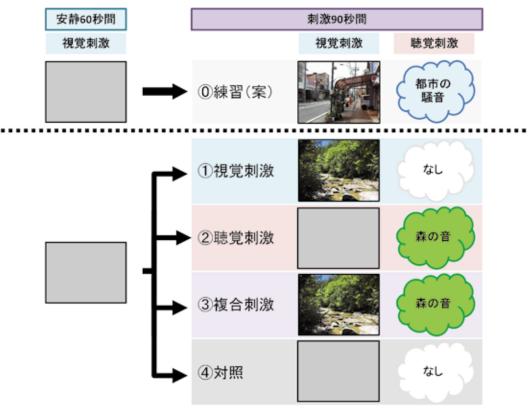
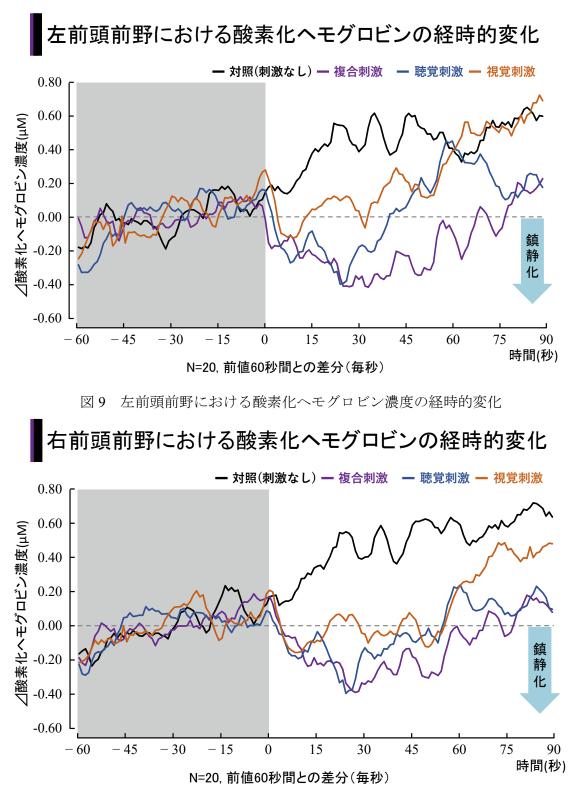


図7 刺激一覧



図8 実験風景

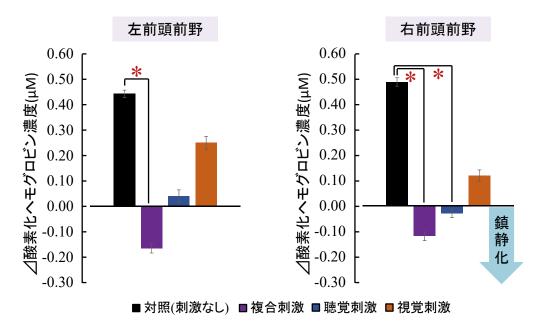
(3) 結果と考察



左右前頭前野における酸素化ヘモグロビン濃度の経時的変化を図9、10に示す。

図 10 右前頭前野における酸素化ヘモグロビン濃度の経時的変化

図9に示すように、左前頭前野における酸素化ヘモグロビン濃度の経時的変化においては、視覚刺激単独、聴覚刺激単独および視覚・聴覚複合刺激において、対照に比べて、低値を示しながら推移した。また、視覚ならびに聴覚単独刺激に比べて、両者の複合刺激においては、最も低く推移しており、複合刺激によって、前頭前野活動が最も鎮静化することが明らかとなった。図10に示すように、右前頭前野活動においても、ほぼ、同様の経時的変化を示した。

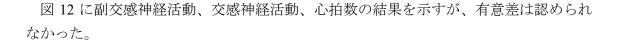


左右前頭前野における酸素化ヘモグロビンの変化

図 11 左右前頭前野における酸素化ヘモグロビン濃度の平均値の比較

図 11 に左右前頭前野における酸素化ヘモグロビン平均濃度を示す。左右前頭前野の 酸素化ヘモグロビン濃度は、複合刺激において、対照に比べて、有意に低下し、前頭前 野活動が鎮静化することが明らかとなった。

N=20, 刺激90秒間の平均値, 平均±標準誤差, *p<0.05, 対照との比較, 対応のあるt検定(Holm補正)



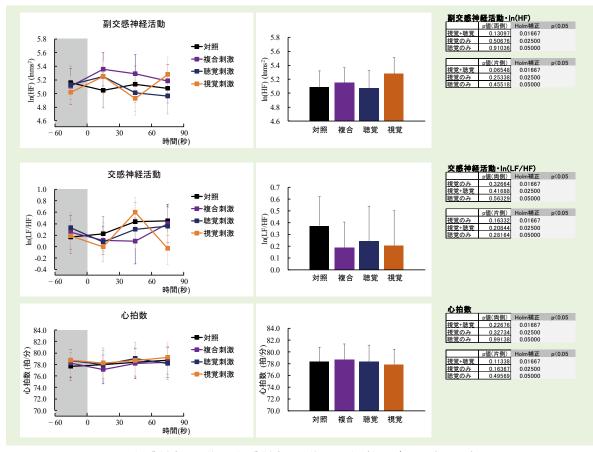


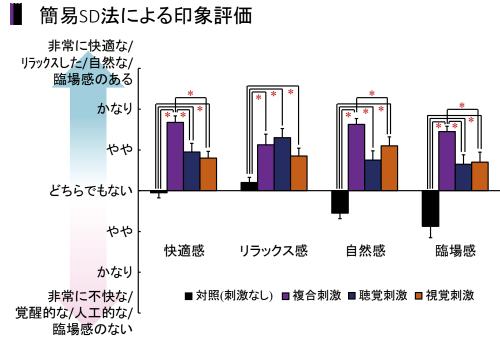
図 12 副交感神経活動、交感神経活動、心拍数の変化(N=20)

図 13 に簡易 SD 法における「快適感」「リラックス感」「自然感」「臨場感」の変化 を示す。

視覚ならびに聴覚単独刺激によって、対照に比べ、「快適感」「リラックス感」「自然 感」「臨場感」が有意に高まることが分かった。

さらに、複合刺激においては、「快適感」「自然感」「臨場感」に関して、視覚単独な らびに聴覚単独に比べて、有意差に高まることが認められた。つまり、視覚・聴覚複 合刺激は、視覚単独刺激および聴覚単独刺激に比べて、快適で、自然で、臨場感があ ると感じられていることが明らかとなった。

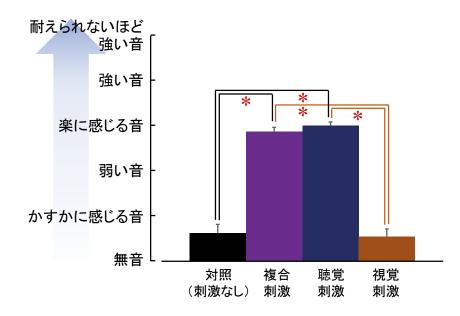
図 14 に主観的音強度の違いを示すが、聴覚単独刺激と聴覚・視覚複合刺激において、ともに「楽に感じる音」と評価され、差異はなかった。



N=20,平均±標準誤差, *p<0.05, ウィルコクソンの符号付順位和検定, Holm補正

図 13 簡易 SD 法における「快適感」「リラックス感」「自然感」「臨場感」の変化

主観的音強度



N=20,平均±標準誤差, *p<0.05, ウィルコクソンの符号付順位和検定, Holm補正

図14 主観的音強度の違い

以上より、森林の視覚・聴覚複合刺激は、対照に比べて、脳前頭前野活動の鎮静化 をもたらすことが明らかとなった。また、視覚単独、聴覚単独、視覚・聴覚複合刺激 においては、対照に比べ、「快適感」「リラックス感」「自然感」「臨場感」が高まり、 さらに、複合刺激は、視覚単独ならびに聴覚単独に比べて、「快適感」「自然感」「臨場 感」を高めることが明らかとなった。 (II) ハイレゾ音と MP3 音が及ぼす影響の違い一室内実験から-

(1) はじめに

前述の複合刺激実験においては、本来の森林の音に近いと考えられるハイレゾ音を用いた。しかし、ハイレゾ音と一般社会において実際に多用されている MP3 音における 聴覚刺激時の生理的・心理的影響の違いについては、現状、明らかになっていない。

そこで、本実験においては、同じ森林音源におけるハイレゾ音と MP3 音の違いを明 らかにすることを目的とした。

(2) 方法

女子大学生 16 名(22.3±2.0 歳)を被験者とし、人工気候室(温度 24℃、湿度 50%、照 度 50 lx)において実施した。被験者情報を図 15 に示す。

年齡

22.3

2.0

0.5

身長

159.8

4.4

1.1

体重

49.6

5.2

1.3

被験者番号	年齡	身長	体重	備考	
sub01	21	159	50		平均
sub02	21	160	51		標準偏差
sub03	_	_	_	×寝たため、削除	標準誤差
sub04	28	159	46		
sub05	21	162	48		
sub06	23	163	60		
sub07	22	163	58		
sub08	21	152	41		
sub09	23	155	48		
sub10	-	-	_	×欠席のため、欠番	
sub11	20	158	46		
sub12	20	165	55		
sub13	22	158	44		
sub14	-	—	_	×欠席のため、欠番	
sub15	23	159	48		
sub16	21	167	55		
sub17	24	166	52		
sub18	22	158	45		
sub19	-	—	_	×欠席のため、欠番	
sub20	-	—	_	×寝たため、削除	
sub21	_	_	_	×欠席のため、欠番	
sub22	24	152.8	46.7		
sub23	_	_	_	×欠席のため、欠番	
sub24	-	_	-	×寝たため、削除	
sub25	—	—	—	×寝たため、削除	

図 15 被験者情報一覧

▶ 測定指標

(1)生理指標

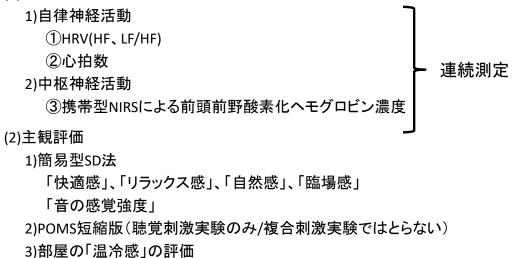
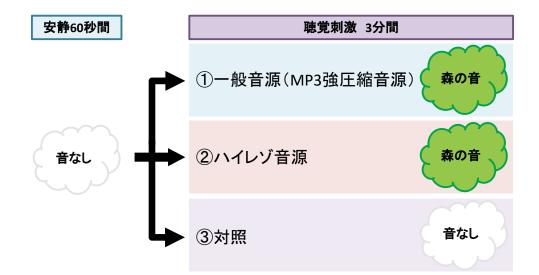


図 16 測定指標





※刺激順は、カウンターバランスをとる。
 ※練習は、実施しない。
 ※音源は、「四万十」とし、0分0秒から3分間呈示する。

図 17 刺激一覧



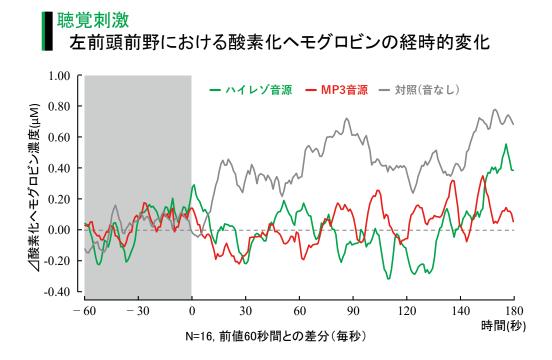
図18 実験風景

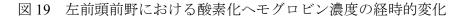
図17に刺激一覧を示し、図18に実験風景を示した。

(3) 結果と考察

図 19 に左前頭前野における酸素化ヘモグロビン濃度の経時的変化を示す。対照に比べて、ハイレゾ音、MP3 音ともに低く推移することが認められた。図 20 に右前頭前野活動の結果を示すが、ほぼ同様の結果であった。

しかし、図 21 に示すように、ハイレゾ音、MP3 音と対照間には有意差はなかった。





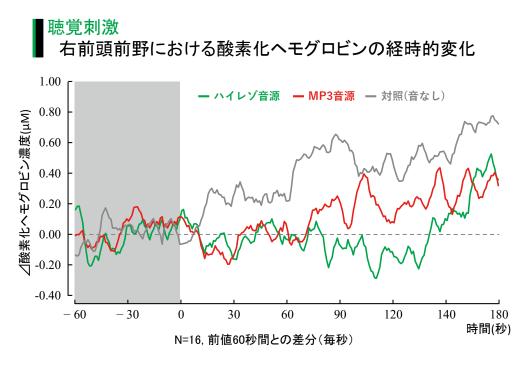
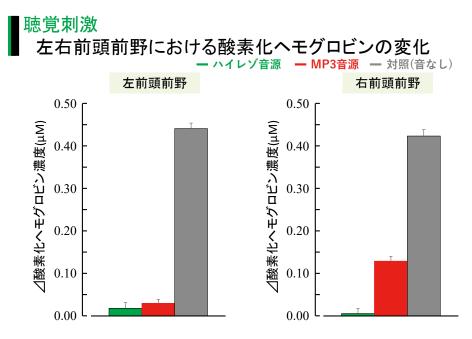
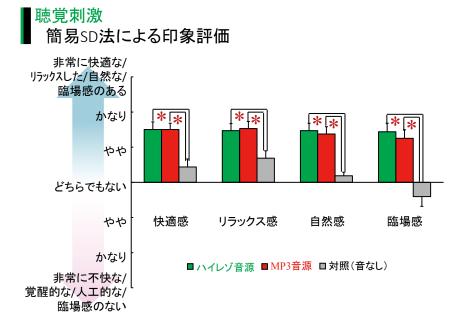


図 20 右前頭前野における酸素化ヘモグロビン濃度の経時的変化



N=16, 刺激180秒間の平均値, 平均±標準誤差, 対応のあるt検定(Holm補正)

図 22 に簡易 SD 法における「快適感」「リラックス感」「自然感」「臨場感」の変化 を示す。ハイレゾ音、MP3 音ともに、対照に比べて、快適で、リラックスし、自然 で、臨場感があると印象されていた。



N=16,平均±標準誤差, *p<0.05, ウィルコクソンの符号付順位和検定, Holm補正

図 22 簡易 SD 法における「快適感」「リラックス感」「自然感」「臨場感」の変化

図 21 左右前頭前野における酸素化ヘモグロビン濃度の平均値の違い

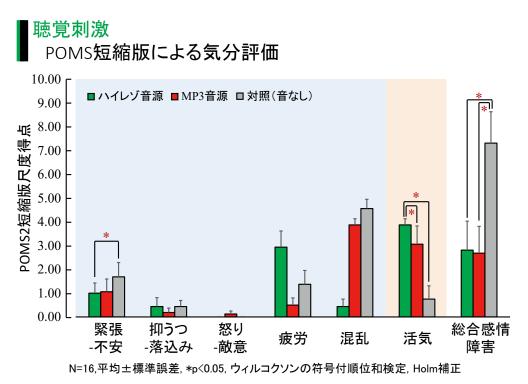
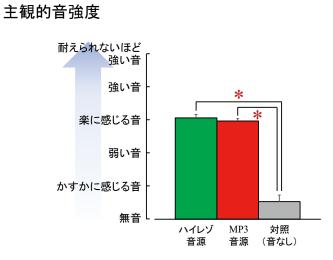


図 23 POMS 短縮版における気分評価

図 23 に POMS 短縮版における気分評価を示す。「総合感情障害」においては、ハ イレゾ音、MP3 ともに有意に気分が改善されることが認められた。ハイレゾ音につい ては、「緊張-不安」尺度が有意に低下し、「活気」尺度が有意に高まることが分かっ た。さらに、「活気」尺度においては、ハイレゾ音は MP3 音に比べて、有意に高いこ とが認められた。

聴覚刺激



N=16,平均±標準誤差, *p<0.05, ウィルコクソンの符号付順位和検定, Holm補正

図24 主観的音強度の違い

54

図 24 に主観的音強度を示すが、ハイレゾ音、MP3 ともに、「楽に感じる音」と評価されており、差異は認められなかった。

(III) うつ病患者を被験者としたビオトープがもたらす効果の解明-フィールド実験から-

(1) 目的

心療内科を主とするクリニックに通院中の男性うつ病患者を被験者として、「樹木と 水の流れを有するビオトープ」による視覚刺激がもたらす生理的・心理的リラックス 効果を明らかにすることを目的とした。

(2) 方法

図25に実験風景を示す。

心療内科を主とするクリニック外壁に施工されているビオトープ(図26左)の視覚 刺激を行った。対照は、対面のビル風景(図26右)とした。

被験者は、クリニックに通院治療中の男性患者、29名とした。平均年齢は43.6歳で あった。



図25 実験風景

被験者が見た景色・被験者情報



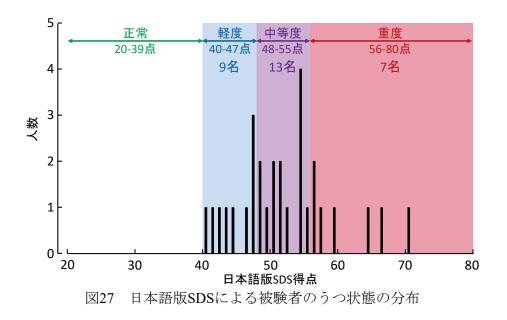
■ 被験者:軽度うつ状態の成人男性29名(平均年齢43.6歳/最年少20歳・最高齢59歳)

	身長	体重	年齡	BMI
mean	168.5	74.0	43.6	26.2
SD	5.4	10.5	8.5	4.3
SE	1.0	2.0	1.6	0.8

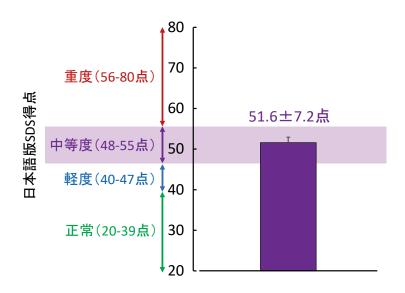
図26 視覚刺激風景と被験者情報

日本語版SDSを用いた被験者のうつ状態の分布を図27に示し、平均値を図28に示 す。「中程度」のうつ状態と評価された。

被験者29名のうつ状態(分布)



被験者29名のうつ状態(平均値)



N=29,平均±標準誤差

図28 日本語版SDSによる被験者のうつ状態得点

実験スケジュール

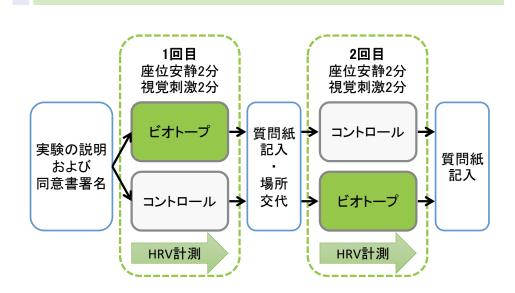


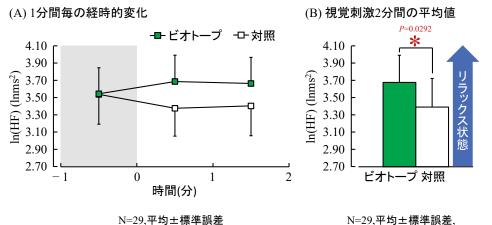
図29 実験スケジュール

図 29 に実験スケジュールを示す。刺激順については、カウンターバランスを取り、 刺激順の影響が出ないように設定した。

(3) 結果と考察

図 30 にビオトープが副交感神経活動に及ぼす影響を示す。通院中うつ病患者は、日常的にストレス状態にあることが知られており、生活の質(QOL)の向上が待たれている。副交感神経活動は、ビオトープの視覚刺激によって、上昇傾向を示し(図 30 左)、 平均値においては、対照に比べて有意に高まることが明らかとなった(図 30 右)。

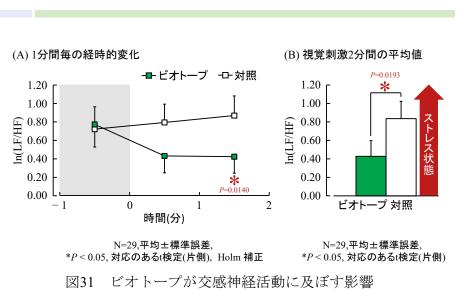
副交感神経活動(In(HF))の変化



*P<0.05,対応のあるt検定(片側)

図30 ビオトープが副交感神経活動に及ぼす影響

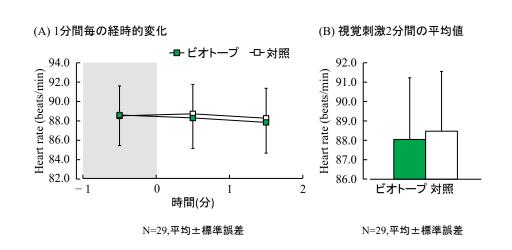
図 31 にビオトープが交感神経活動に及ぼす影響を示す。ビオトープの視覚刺激によって有意に低下し(図 31 左)、平均値においても、対照に比べて有意に低下することが明らかとなった(図 31 右)。



交感神経活動(In(LF/HF))の変化

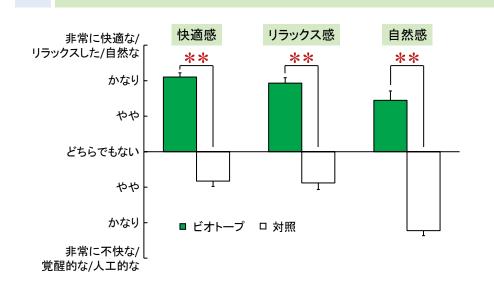
59

図32にビオトープが心拍数に及ぼす影響を示すが、差異は認められなかった。



心拍数の変化

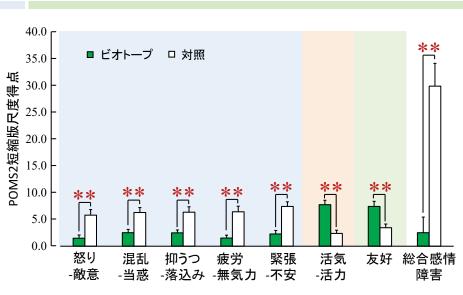
図32 ビオトープが心拍数に及ぼす影響



簡易SD法

N=29,平均±標準誤差,**P<0.01,ウィルコクソンの符号付順位和検定(片側)

図33 ビオトープが「快適感」「リラックス感」「自然感」の及ぼす影響 図33 にビオトープが「快適感」「リラックス感」「自然感」の及ぼす影響を示す。ビ オトープはうつ病患者に対して、快適で、リラックスし、自然な印象をもたらすことが 明らかとなった。



POMS2短縮版

N=29,平均±標準誤差,**P<0.01,ウィルコクソンの符号付順位和検定(片側)

図 34 POMS 短縮版における気分評価

図 34 に示すように、ビオトープの視覚刺激によって、「怒りー敵意」「混乱-当惑」 「抑うつ-落込み」「疲労-無気力」「緊張-不安」「活気-活力」「友好」「総合感情尺 度」のすべてにおいて、有意に気分が改善することが明らかとなった。

おわりに

室内実験における森林の視覚・聴覚複合刺激は、脳前頭前野活動の鎮静化を生じる ことが明らかとなった。また、視覚単独、聴覚単独、視覚・聴覚複合刺激において は、対照に比べ、「快適感」「リラックス感」「自然感」「臨場感」が高まり、さらに、 複合刺激は、視覚単独ならびに聴覚単独に比べて、「快適感」「自然感」「臨場感」を高 めることが明らかとなった。

フィールド実験における病院外壁ビオトープ視覚刺激実験においては、1)生理評価 においては、リラックス時に高まる副交感神経活動を亢進させ、ストレス時に高まる交 感神経活動を抑制すること、2)主観評価においては、「快適感」「リラックス感」「自然 感」を高め、POMSにおいても気分の改善をもたらすことが明らかとなった。 上記の「室内実験」「フィールド実験」から、自然由来の刺激は、生理的ならびに心 理的リラックス効果を持つことが認められた。

本研究は以下のメンバーの協力の元に実施された(五十音順)。 池井晴美((国研)森林研究・整備機構森林総合研究所) 榎本誠也((株)JVCケンウッド・ビクターエンタテインメント) 嵯峨崎 泰子(医療法人社団ユメイン 野崎クリニック) 宋チョロン(千葉大学環境健康フィールド科学センター) 野崎 英樹(医療法人社団ユメイン 野崎クリニック)

平成30年度

森林浴による健康増進等に関する調査研究

報告書

千葉大学環境健康フィールド科学センター

宮崎良文

平成 30 年度目次

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はじめに

森林セラピー研究は、日本において1990年代に始まり、ここ十数年で多くの生理デ ータが蓄積されてきた。森林セラピー実験には、森林等の現場で実施する「フィール ド実験」と「人工気候室内実験」が存在し、これまで、本研究課題実行者が中心とな り、生理的リラックス効果に関するデータの多くを提出してきた。これは世界に類を 見ない科学的データの蓄積である。

一方、最近、これまでに蓄積されてきた健常人に対する影響評価に加えて、精神疾 患患者等の高ストレス状態の方々に対する生理的影響に関心が集まっている。また、 自然由来の聴覚刺激実験は、他の感覚刺激実験に比べて、データがほとんど提出され ていないという現状がある。

そこで、今年度は、1)「フィールド実験」においては、通院中のうつ病患者を被験 者として、待合室における木質壁の生理的リラックス効果を解明すること、2)「人工 気候室内実験」においては、森林ハイレゾ音の「聴覚刺激」がもたらす生理的リラッ クス効果を都市音と比較することにより解明することを目的とした。 I 平成 30 年度の概要

(1)研究内容

1)クリニック内木質待合室がもたらす生理的リラックス効果の解明

通院中の男性うつ病患者を被験者として、クリニック内の木質待合室がもたらす 生理的・心理的リラックス効果を明らかにすることを目的とする。(以下、「木質 内装壁実験」)

2)森林内ハイレゾリューション音源のヘッドホンによる聴覚刺激実験

20代女性を被験者とした2年目(H29年度)の実験において、ハイレゾリューショ ン音源を使用した自然由来の聴覚刺激実験を実施したが、人工気候室内の暗騒音が 大きな影響をもたらすことが分かった。今回は、この問題を解消して、ハイレゾリ ューション音源による影響を明らかにするため、同じく20代女性を被験者として、 ヘッドホン使用による追加実験を実施する。(以下、「聴覚刺激実験」)

(2)年間スケジュール

平成30年度の研究スケジュールを以下に示す。

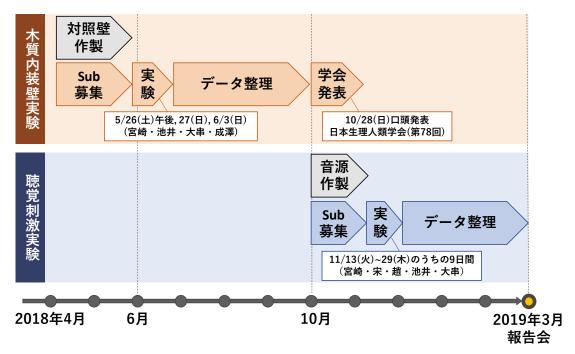


図1 研究スケジュール

なお、研究担当者は以下の通りである。

1) 木質内装壁実験

宮崎良文、池井晴美((国研)森林研究・整備機構森林総合研究所)、野崎英樹(医療法人社団ユメイン 野崎クリニック)・嵯峨崎泰子(医療法人社団ユメイン 野崎クリニック)

 2) 聴覚刺激実験
 宮崎良文、宋チョロン、趙炫珠(千葉大学環境健康フィールド科学センター)、
 池井晴美((国研)森林研究・整備機構森林総合研究所)、小林宏光(石川県立看護 大学)

II 木質内装壁実験

1.方法

(1)目的

木質内装壁の視覚刺激が軽度うつ状態者にもたらす生理的・心理的影響を明らかにすることである。

(2)被験者募集情報

1)人数

2名1組で実施

①午前: 09:40-10:20 2名, 10:20-11:00 2名 11:00-11:40 2名
②午後: 13:20-14:00 2名, 14:00-14:40 2名 14:40-15:20 2名, 15:20-16:00 2名

2)属性

①20代~30代(20~39歳)の軽度うつ状態の男性 ※主として前回参加者
 ☑慢性鼻炎・喘息を持っていない方
 ☑実験中、眠らない方
 ☑不整脈でない方
 ☑煙草を吸わない方

3) 拘束時間:合計約40分程度の拘束

 ①控え室での説明:約15分程度

・実験の説明(倫理審査委員会説明書・同意書署名)

・パーソナリティ質問紙 & うつ状態評価質問紙記入

②実験室での計測:約20分程度

4) 被験者料: 10,000円(交通費含む)

(3)被験者と測定指標

実験場所、被験者情報、生理指標、主観評価、検定法を以下に記す。

■場所 医療法人社団ユメイン野崎クリニック内の待合室

■ 被験者 通院男性うつ病患者24名

■ 生理指標

自律神経活動	心拍変動性(HRV)によるHF, LF/HF
	心拍数

■ 主観評価

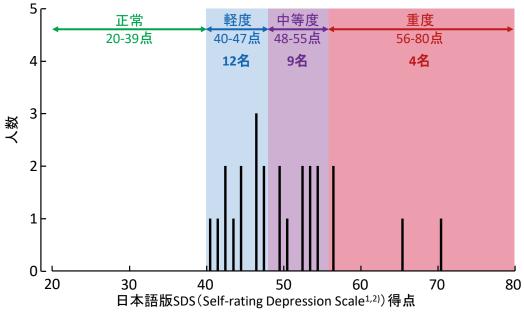
簡易SD法	快適感, リラックス感, 自然感
POMS2短縮版	怒り-敵意, 混乱-当惑, 抑うつ-落込み
	疲労-無気力,緊張-不安,活気-活力,友好

有意差の検定

生理指標	対応のあるt検定(Holm補正)
主観評価	ウィルコクソンの符号付順位和検定

図2 実験場所、被験者情報、生理指標、主観評価、検定法

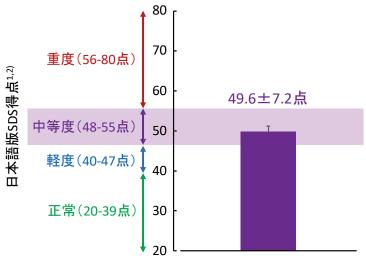
被験者 25 名のうつ状態の分布を以下に記す。



1) Zung, WWK (1965) A self-rating depression scale. Archives of General Psychiatry,12,63-70. 2) 福田一彦,小林重雄.自己評価式抑うつ尺度の研究...精神経誌 1973; 75: 673-679.

図3 うつ状態の分布

被験者 25 名のうつ状態の平均値を以下に記す。 日本語版 SDS 得点は 49.6 点であり、中程度(48~55 点)のうつ状態であった。



N=25,平均±標準誤差

1) Zung, WWK (1965) A self-rating depression scale. Archives of General Psychiatry,12,63-70. 2) 福田一彦,小林重雄.自己評価式抑うつ尺度の研究.. 精神経誌 1973; 75: 673-679.

図4 うつ状態の平均値

心拍変動性(HRV)による副交感・交感神経活動計測の原理を以下に示す。最大エント ロピー法を用い、HFを副交感神経活動の指標、LF/HFを交感神経活動の指標とした。

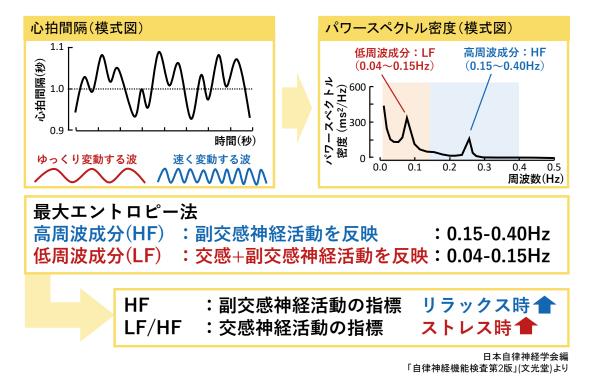
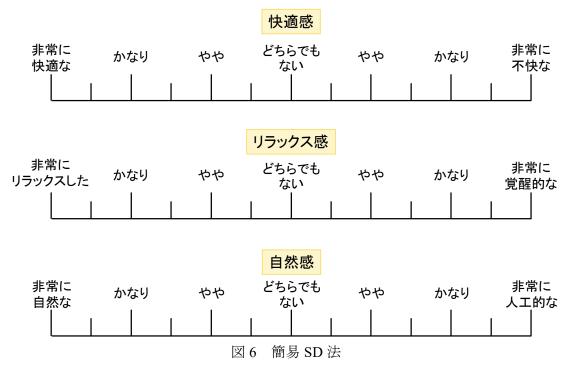


図5 副交感・交感神経活動計測の原理

簡易 SD 法による快適感、リラックス感、自然感の質問紙を以下に記す。



気分プロフィール検査(POMS)2 短縮版の質問紙を以下に記す。



図7 気分プロフィール検査(POMS)2 短縮版

(4)実験プロトコル

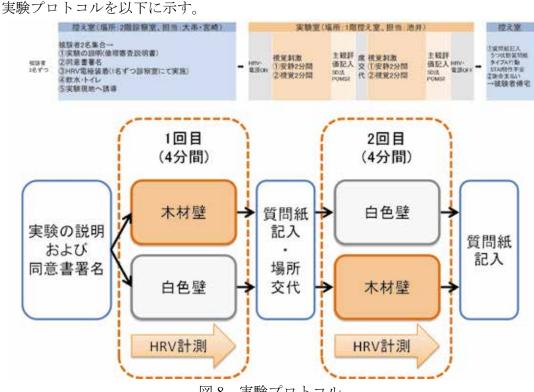


図8 実験プロトコル

(5)実験室の設定と実験風景

実験室の設定を以下に示す。

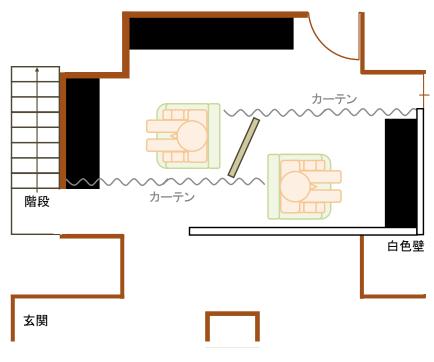


図9 実験室の設定

木質待合室を以下に示す。

施工は以下の通りである。

デザイン・施工:株式会社グラマラス

広さ:横3360×奥行3050×高さ2400mm(約6.8畳,20席)

内装材:ヤチダモ(Fraxinus mandshurica)材、オイル仕上げ(一部挽き板複合材)

加工法:4 種類のなぐり加工(KIRISAME, SAZANAMI, NARUTO, KIKKOU, 株式会

社ノスタモ)

施工完了日:2016年5月中旬



図 10 木質待合室

刺激として用いた待合室の木質壁を示す。



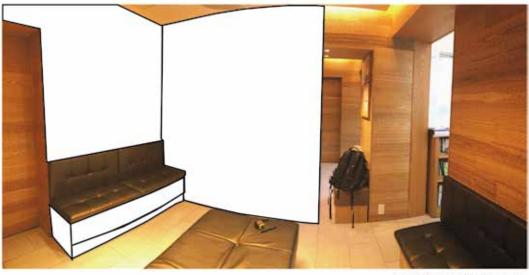
【撮影】池井(2018年3月17日)

図11 刺激として用いた待合室木質壁

白色壁(対照)のイメージを以下に示す。

白色壁のイメージ

白色壁は、安アパートの壁面に使用されているような安価な印象のものとする。 壁面備え付けのソファーの下部においても、木材が隠れるよう白色壁を設置する。



【撮影】池井(2018年3月17日)



図 12 対照として用いた白色壁のイメージ 対照壁の作成風景を以下に示す。

図13 対照壁の作成風景

実験室(木質待合室)の配置を以下に示す。

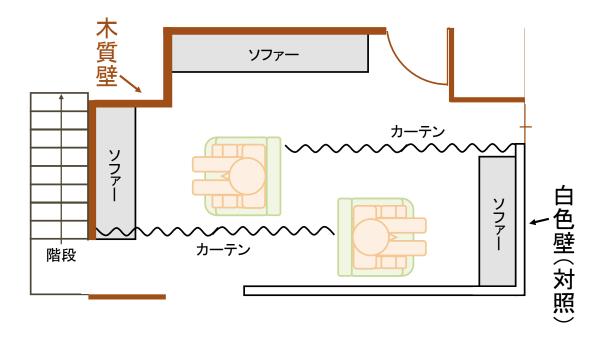
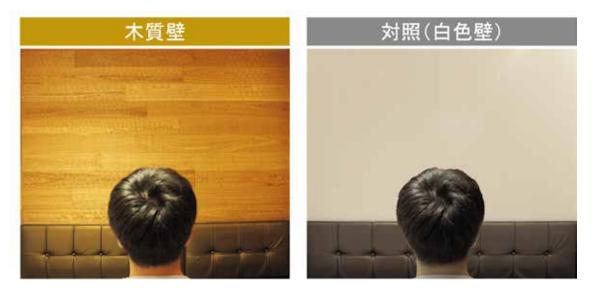


図14 実験室(木質待合室)の配置

実験風景と被験者情報を以下に示す。



■ 被験者:通院男性うつ病患者24名(最年少27歳・最高齢60歳)

	身長	体重	年齡	BMI
mean	169.8	76.7	44.1	26.7
SD	7.1	14.6	7.4	5.4
SE	1.5	3.0	1.5	1.1

図15 実験風景と被験者情報

2.結果

研究の背景を以下に示す。

木材が人にもたらすリラックス効果については、経験的に知られており、近年、健常 者を用いた生理データが蓄積されつつある。

しかし、うつ病患者等の高ストレス群を対象とした研究は存在しない。

木材が人にもたらすリラックス効果は経験的



うつ病患者等の高ストレス群を対象としたデータの欠如

図16 研究の背景

(1)生理指標

待合室の木質壁が副交感神経活動にもたらす影響を以下に記す。

木質壁を見た場合、対照(白色壁)に比べて、有意に副交感神経活動が高まることが 示され、生体がリラックス状態になることが分かった。

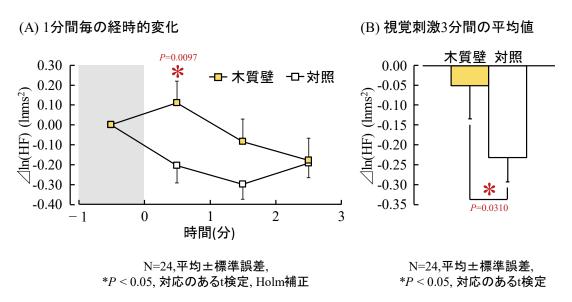


図 17 待合室木質壁の視覚刺激が副交感神経活動にもたらす影響 待合室の木質壁が心拍数にもたらす影響を以下に記す。

木質壁を見た場合、対照(白色壁)に比べて、有意に心拍数が低下することが示され、 生体がリラックス状態になることが分かった。

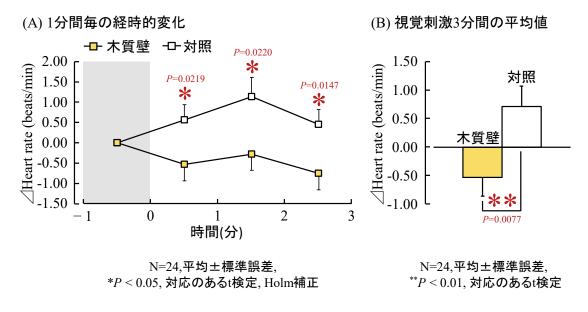


図18 待合室木質壁の視覚刺激が心拍数にもたらす影響

待合室の木質壁が交感神経活動にもたらす影響を以下に記す。

木質壁を見た場合、対照(白色壁)に比べて、交感神経活動が低下する傾向にあることが示された。

(A) 1分間毎の経時的変化

(B) 視覚刺激3分間の平均値

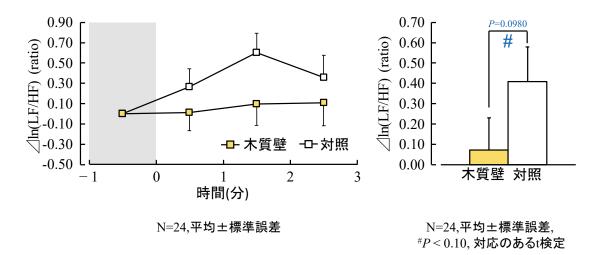
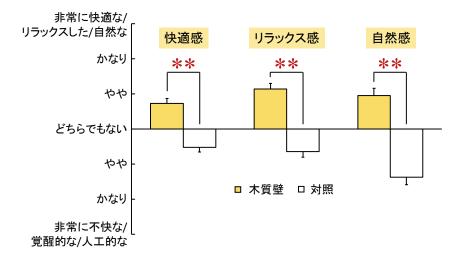


図 19 待合室木質壁の視覚刺激が交感神経活動にもたらす影響

(2)主観評価

待合室の木質壁が簡易 SD 法(快適感、リラックス感、自然感)にもたらす影響を以下に記す。

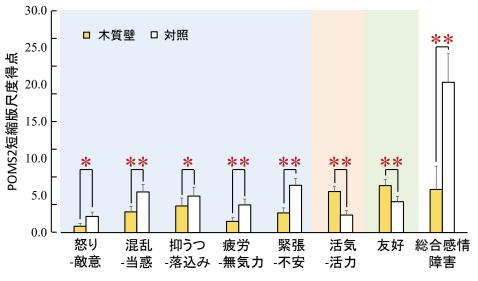
木質壁を見た場合、対照(白色壁)に比べて、有意に「快適感」「リラックス感」「自然感」が高まることが示された。



N=24,平均±標準誤差,**P < 0.01, ウィルコクソンの符号付順位和検定

図 20 待合室木質壁の視覚刺激が「快適感」「リラックス感」「自然感」にもたらす影響

待合室の木質壁が気分プロフィール検査(POMS2)にもたらす影響を以下に記す。 木質壁を見た場合、対照(白色壁)に比べて、有意に「怒り-敵意」「混乱-当惑」「抑 うつ-落込み」「疲労-無気力」「緊張-不安」が有意に低下し、「活気-活力」「有効」が高ま ることが示された。「総合感情障害」も有意に低下した。



N=24,平均±標準誤差,*P<0.05,**P<0.01,ウィルコクソンの符号付順位和検定

図 21 待合室木質壁の視覚刺激が気分プロフィール検査(POMS2)にもたらす影響

結論として、木質内装壁の視覚刺激は、対照(白色壁)と比較し、

(1)生理指標において

- 1) 心拍変動性 ln(HF)が有意に上昇すること
- 2) 心拍数が有意に低下すること
- 3) 心拍変動性 ln(LF/HF)が低下する傾向にあること

(2)主観評価において

1) 快適感、リラックス感、自然感が有意に高まること

2) 気分状態が有意に改善されること

が示された。

医療機関待合室の木質内装は、通院うつ病患者に対して生理的・心理的リラックス効果をもたらすことが明らかとなった。

III 聴覚刺激実験

1.方法

(1)目的

- 1) 森林と都市の聴覚刺激実験 森林由来のハイレゾリューション音が及ぼす生理的効果を明らかにする。
- 2) 森林由来のハイレゾ音と MP3 音の比較実験 質の異なる森林由来の聴覚刺激を受けた際の生理応答の違いを明らかにする。

(2)被験者募集情報

1)人数

①午前 : 09:00-10:30 1名, 10:30-12:00 1名

- ②午後 : 13:00-14:30 1名, 14:30-16:00 1名
- 1日4名×6日間
- → +1 日 2 名×3 日間=合計 30 名
- 2)20代(20~29歳)の女子大学生・大学院生
 - ☑実験日に月経期でない方
 - ☑慢性鼻炎・喘息を持っていない方 ☑不整脈でない方

☑正常な聴覚をもっている方
 ☑不整脈でない方
 ☑煙草を吸わない方

- 3) 拘束時間:合計約1時間30分程度の拘束
 - ①控え室:30分程度

☑実験中、眠らない方

- ・実験の説明(倫理審査委員会説明書・同意書署名)
- ・パーソナリティ質問紙記入
- ②実験室内:約60分程度

4)被験者料: 6,000 円+交通費

(3)被験者

20代の女子大学生・大学院生 29名

(4)聴覚刺激

1)森林と都市の聴覚刺激実験

①森の聴覚刺激:戸隠高原・ハイレゾ (平均音圧:48.6dB)
 ②対照:都市の聴覚刺激:街頭音_渋谷・ハイレゾ (平均音圧:51.5dB)

2) 森林由来のハイレゾ音と MP3 音の比較実験
 ①ハイレゾ:四万十 (平均音圧: 49.2dB)
 ②MP3:四万十 (平均音圧: 49.0dB)

(5)測定指標

以下に測定指標を示す。



図 22 測定指標

(6)実験プロトコル

1) 森林音と都市音の聴覚刺激実験

以下に実験プロトコルを示す。

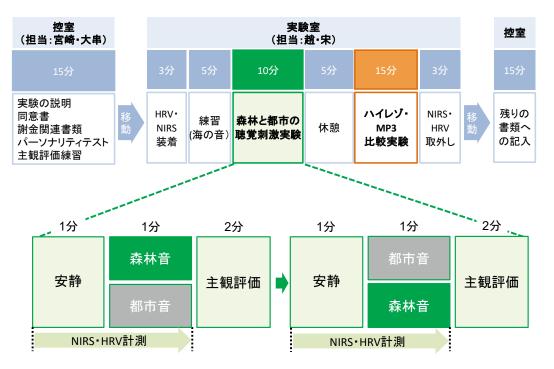


図23 森林音と都市音の聴覚刺激実験プロトコル

2) 森林由来のハイレゾ音と MP3 音の比較実験

以下に、森林由来のハイレゾ音と MP3 音の聴覚刺激における比較実験プロトコルを示す。

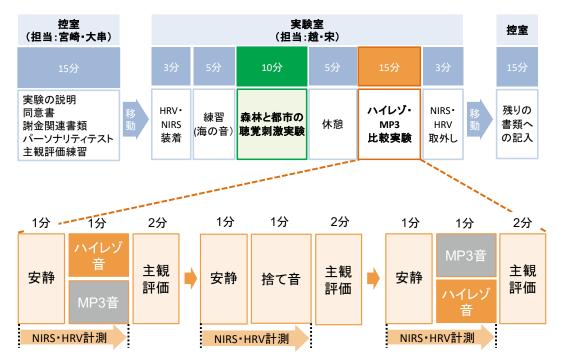


図 24 森林ハイレゾ音と MP3 音の聴覚刺激比較実験プロトコル

(7)人工気候室内の設定と実験風景

以下に、人工気候室内の設定を示す。

温度 25℃、湿度 50%、照度 200Lx にて実施した。

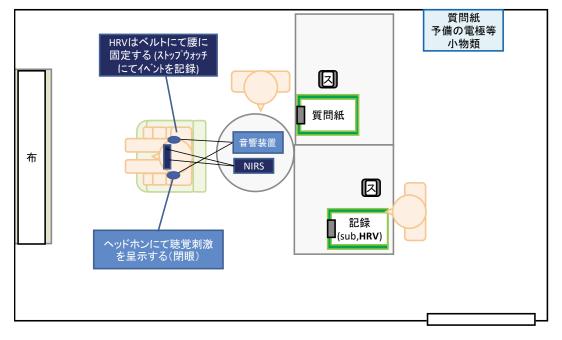


図 25 人工気候室内の設定

以下に、実験風景を示す。



図 26 実験風景

2.結果

(1)森林と都市の聴覚刺激実験

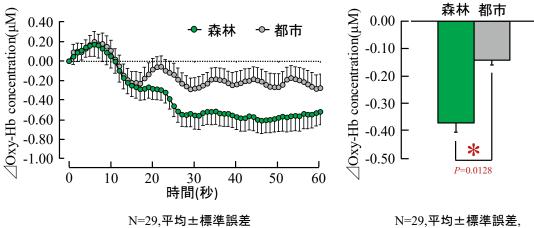
1)生理指標

以下に、ハイレゾ森林音とハイレゾ都市音の聴覚刺激が右前頭前野活動に及ぼす影響 を示す。

森林ハイレゾ音の聴覚刺激によって、右前頭前野活動が有意に鎮静化することが明ら かとなった。

(A)1秒間毎の経時的変化

(B) 聴覚刺激1分間の平均値

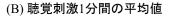


*P < 0.05, 対応のあるt検定(片側)

図 27 ハイレゾ森林音とハイレゾ都市音の聴覚刺激が右前頭前野活動にもたらす影響 以下に、ハイレゾ森林音とハイレゾ都市音の聴覚刺激が左前頭前野活動に及ぼす影響 を示す。

森林ハイレゾ音の聴覚刺激によって、左前頭前野活動が鎮静化する傾向になることが わかった。

(A) 1秒間毎の経時的変化



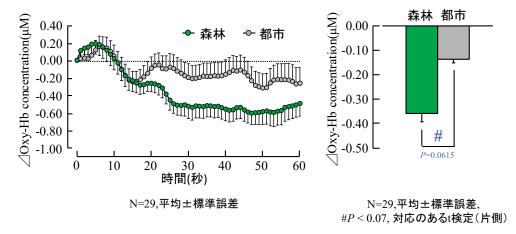
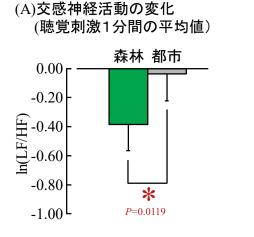
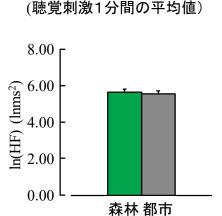


図 28 ハイレゾ森林音とハイレゾ都市音の聴覚刺激が左前頭前野活動にもたらす影響

以下に、ハイレゾ森林音とハイレゾ都市音の聴覚刺激が交感神経活動と副交感神経活動に及ぼす影響を示す。

森林ハイレゾ音の聴覚刺激によって、交感神経活動が有意に低下し、ストレス状態が 抑制されることが明らかとなった。副交感神経活動においては、差異はなかった。





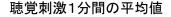
(B)副交感神経活動の変化

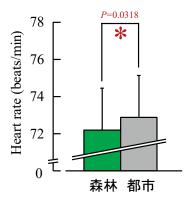
N=29,平均±標準誤差, *P < 0.05,対応のあるt検定(片側)

N=29,平均±標準誤差, 対応のあるt検定(片側)

図 29 ハイレゾ森林音とハイレゾ都市音の聴覚刺激が 交感神経活動と副交感神経活動にもたらす影響

以下に、ハイレゾ森林音とハイレゾ都市音の聴覚刺激が心拍数に及ぼす影響を示す。 森林ハイレゾ音の聴覚刺激によって、心拍数が有意に低下し、リラックスすることが 明らかとなった。



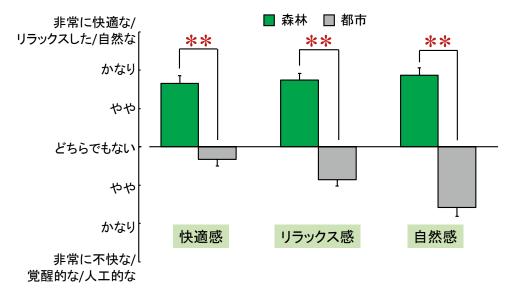


N=29,平均±標準誤差, *P < 0.05,対応のあるt検定(片側)

図 30 ハイレゾ森林音とハイレゾ都市音の聴覚刺激が心拍数にもたらす影響

2)主観評価

以下に、ハイレゾ森林音とハイレゾ都市音の聴覚刺激が心拍数に及ぼす影響を示す。 森林ハイレゾ音の聴覚刺激によって、「快適感」「リラックス感」「自然感」が有意に高 まることが明らかとなった。

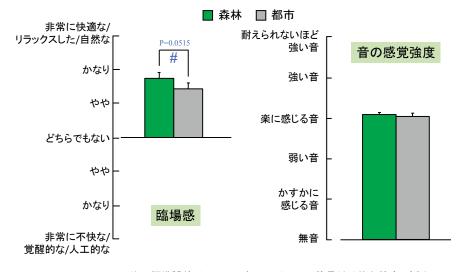


N=29,平均±標準誤差,**P < 0.01, ウィルコクソンの符号付順位和検定(片側)

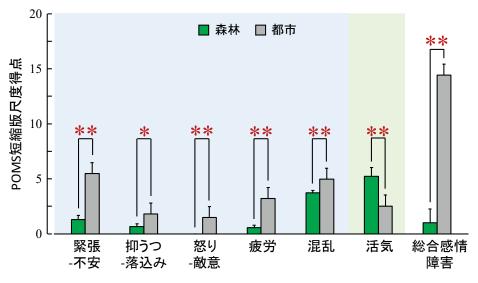
図 31 ハイレゾ森林音とハイレゾ都市音の聴覚刺激が

「快適感」「リラックス感」「自然感」にもたらす影響

以下に、ハイレゾ森林音とハイレゾ都市音の聴覚刺激が「臨場感」と「感覚強度」に 及ぼす影響を示す。森林ハイレゾ音の聴覚刺激において、「感覚強度」に差異がないこ とが認められた。



N=29,平均±標準誤差,*P<0.06,ウィルコクソンの符号付順位和検定(片側) 図 32 ハイレゾ森林音とハイレゾ都市音の聴覚刺激が 「臨場感」と「感覚強度」にもたらす影響 以下に、ハイレゾ森林音とハイレゾ都市音の聴覚刺激が気分プロフィール検査 (POMS)に及ぼす影響を示す。森林ハイレゾ音の聴覚刺激によって、「緊張-不安」「抑 うつ-落込み」「怒り-敵意」「疲労」「混乱」が有意に低下し、「活気」が高まることが示 された。「総合感情障害」も有意に低下した。



N=29,平均±標準誤差,*P<0.05,**P<0.01,ウィルコクソンの符号付順位和検定(片側)

結論として

森林の聴覚刺激は、都市の聴覚刺激と比較し、

(1)生理指標において

- 1) 右前頭前野活動が有意に低下すること
- 2) 心拍変動性 ln(LF/HF)が有意に低下すること
- 3) 心拍数が有意に低下すること
- (2)主観評価において
- 1) 快適感、リラックス感、自然感が有意に高まること
- 2) 気分状態が有意に改善されること
- が分かった。

森林の聴覚刺激は、生理的・心理的リラックス効果をもたらすことが明らかとなった。

図 33 ハイレゾ森林音とハイレゾ都市音の聴覚刺激が気分プロフィール検査 (POMS) にもたらす影響

(2) 森林由来のハイレゾ音と MP3 音の比較実験

1)生理指標

以下に、森林由来のハイレゾ音と MP3 音が左前頭前野活動にもたらす影響を示す。 ハイレゾ音によって、前頭前野活動が鎮静化するとの仮説の元に実施したが、MP3 音で 鎮静化し、ハイレゾ音においては変化しないことが明らかとなった。その理由は、不明 であるが、日常的に聴取している音が MP3 音であるため、聞き慣れていることが影響 したと予想している。

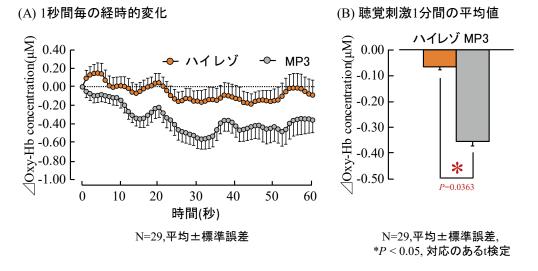


図 34 森林由来のハイレゾ音と MP3 音が左前頭前野活動にもたらす影響

以下に、森林由来のハイレゾ音と MP3 音が右前頭前野活動にもたらす影響を示す。 ハイレゾ音によって、左前頭前野活動と同様に、MP3 音で鎮静化し、ハイレゾ音におい ては変化しないことが明らかとなった。

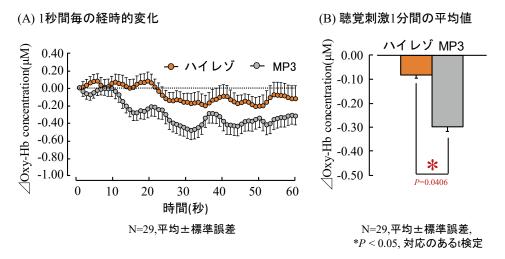
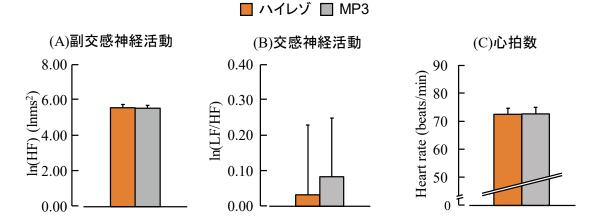


図 35 森林由来のハイレゾ音と MP3 音が右前頭前野活動にもたらす影響

以下に、森林由来のハイレゾ音と MP3 音が自律神経活動にもたらす影響を示す。副 交感神経活動、交感神経活動、心拍数ともに差異は認められなかった。

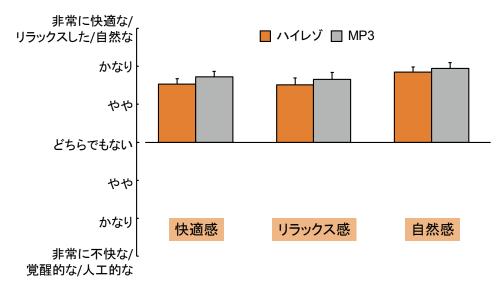


聴覚刺激1分間の平均値

N=29,平均±標準誤差、対応のあるt検定

図 36 森林由来のハイレゾ音と MP3 音が副交感神経活動、交感神経活動、心拍数に もたらす影響

以下に、森林由来のハイレゾ音と MP3 音が「快適感」「リラックス感」「自然感」に もたらす影響を示す。「快適感」「リラックス感」「自然感」ともに差異は認められなか った。



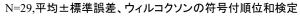
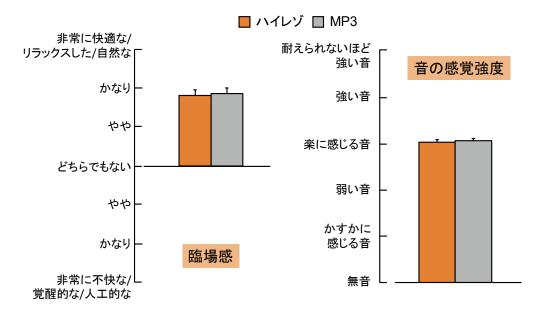


図 37 森林由来のハイレゾ音と MP3 音が「快適感」「リラックス感」「自然感」に もたらす影響

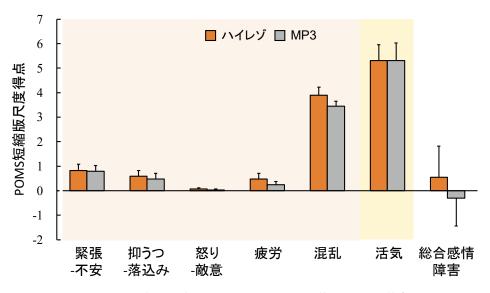
以下に、森林由来のハイレゾ音と MP3 音が「臨場感」と「感覚強度」にもたらす影響を示す。「臨場感」「感覚強度」ともに差異は認められなかった。



N=29,平均±標準誤差,ウィルコクソンの符号付順位和検定

図 38 森林由来のハイレゾ音と MP3 音が「臨場感」と「感覚強度」にもたらす影響

以下に、森林由来のハイレゾ音と MP3 音が気分プロフィール検査(POMS)にもた らす影響を示す。「緊張-不安」「抑うつ-落込み」「怒り-敵意」「疲労」「混乱」「活気」 「総合感情障害」ともに差異は認められなかった。



N=29,平均±標準誤差,*P<0.05,ウィルコクソンの符号付順位和検定

図 39 森林由来のハイレゾ音と MP3 音が気分プロフィール検査 (POMS) に もたらす影響

さらに、タイプ A 行動パターンを用いたパーソナリティの違いによる分析を行った。

以下に、タイプA行動パターンの説明を記す。

・FriedmanとRosenman(1961)によるタイプA行動パターンの特徴としては

- 1) 強い競争心
- 2) 時間的切迫感
- 3) 短気4) 精神的·肉体的過激性

などが挙げられる。

日頃のあなた自身の生活や態度について	(KG式日常生活質問紙)
あてはまるように、右の数字に〇をつけて下さい。	
朝はだいたいすっきり起きられる・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・	・・・ はい ? いいえ
すんだことをくよくよと考えることが多い・・・・・・・・・	・・・・はい ? いいえ
話すとき身振りが多い・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・	・・・ はい ? いいえ
いつも何かしていないと落ち着かない・・・・・・・・・・	・・・ はい ? いいえ

図 40 タイプ A 行動パターン

以下に被験者のタイプA行動パターンを記す。

被験者番号				利手	パーソナリティ	
	身長	体重	年齢		TypeA行動バターン	STAI特性不安
sub01	160	48	23	右	typeA	normal
sub02	157	45	20	右	typeB	high
sub03	168	49	27	右	typeB	normal
sub04	146	41	28	右	typeA	high
sub05	150	44	22	右	typeA	normal
sub06	166	64	24	右	typeA	high
sub07	168	55	21	右	typeA	high
sub08	157	51	24	右	typeB	high
sub09	146	44	20	右	typeB	high
sub10	159	57	21	右	typeB	high
sub11	162	47	21	右	typeB	normal
sub12	157	49	21	右	typeB	high
sub13	160	55	21	右	typeA	normal
sub14	161	55	21	右	typeB	normal
sub15	160	48	22	右	typeB	high
sub16	160	42	25	右	typeA	high
sub17	164	53	23	右	typeA	high
sub18	156	44	21	右	typeB	high
sub19	165	53	24	右	typeA	normal
sub20	166	60	25	右	typeA	normal
sub21	164	56	20	右	typeB	high
sub22	158	45	21	右	typeB	high
sub23	156	45	20	右	typeB	high
sub24	159	52	20	右	typeA	high
sub25					欠席	
sub26	157	44	22	右	typeB	high
sub27	150	50	20	右	typeB	high
sub28	158	51	24	右	typeB	normal
sub29	153	42	22	右	typeA	high
sub30	158	45	23	左	typeB	high
	• •	44.45	A- #A	1		· • •
平均值	身長 158.6	体重 49.4	年齢 22.3	-	TypeA行動/ TypeA群	バターン 12
<u>平均112</u> SD	5.8	49.4 5.8	22.3		ТуреА	12
SE	1.1	1.1	0.4	1	STAI特性	
36	1.1	1.1	0.4	1	STATAT	

被験者:成人女子大学生29名

 STAI特性不安

 高不安群
 20

 普通・低不安群
 9

図 41 被験者のタイプ A 行動パターン分類

以下に、タイプA群における森林由来のハイレゾ音とMP3音が左前頭前野活動に もたらす影響を示す。MP3音において、左前頭前野活動が大きく鎮静化することが明 らかとなった。

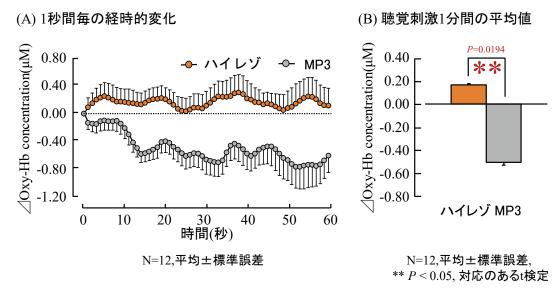


図 42 タイプ A 群における森林由来のハイレゾ音と MP3 音が左前頭前野活動に もたらす影響

以下に、タイプA群における森林由来のハイレゾ音とMP3音が右前頭前野活動に もたらす影響を示す。左前頭前野活動と同様に、MP3音において、右前頭前野活動が 大きく鎮静化することが明らかとなった。

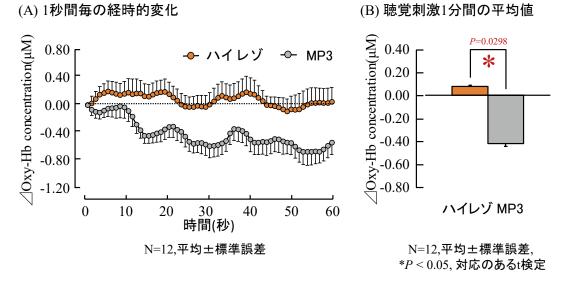


図 43 タイプ A 群における森林由来のハイレゾ音と MP3 音が右前頭前野活動に もたらす影響

92

以下に、タイプB群における森林由来のハイレゾ音とMP3音が左前頭前野活動にも たらす影響を示す。MP3音とハイレゾ音間に差異は認められなかった。

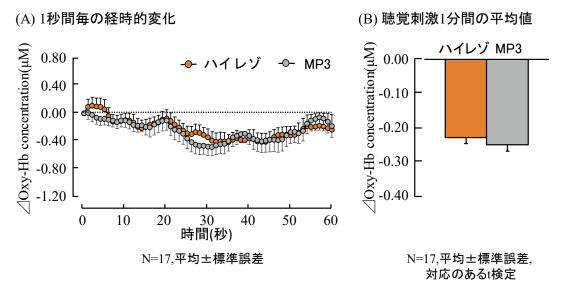


図 44 タイプ B 群における森林由来のハイレゾ音と MP3 音が左前頭前野活動に もたらす影響

以下に、タイプB群における森林由来のハイレゾ音とMP3音が右前頭前野活動にも たらす影響を示す。左前頭前野活動と同様に、MP3音とハイレゾ音間に差異は認めら れなかった。

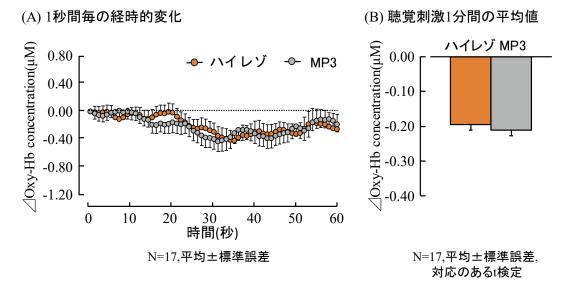
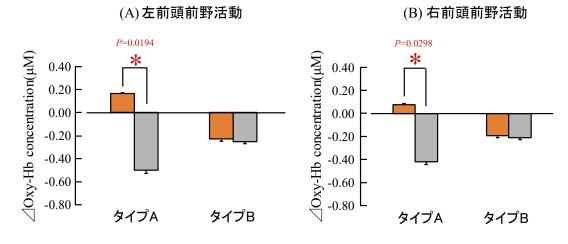


図 45 タイプ B 群における森林由来のハイレゾ音と MP3 音が右前頭前野活動に もたらす影響

以下に、タイプA群とB群における森林由来のハイレゾ音とMP3音が左右前頭前 野活動にもたらす影響を纏めて示す。左右前頭前野活動ともに、タイプA群では差異 が認められるが、タイプB群では差異がないことが明らかとなった。



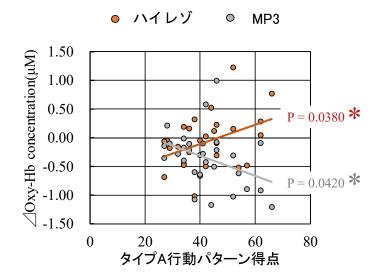
🔲 ハイレゾ 🛛 🔲 MP3

タイプA群:N=12,タイプB群:N=17, 平均±標準誤差,*P<0.05,対応のあるt検定

図 46 タイプA 群とB 群における森林由来のハイレゾ音と MP3 音が左右前頭前野 活動にもたらす影響

以下に、タイプA行動パターン得点と左前頭前野活動の関係を示す。

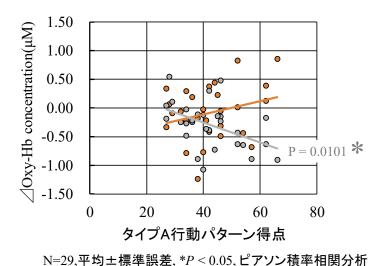
得点の上昇とともに、ハイレゾ音においては活動が上昇し、MP3 音においては活動 が低下することが分かった。図 42 に示すように、44 点以上のタイプ A 群において、 ハイレゾ音と MP3 音間に差異が認められている。



N=29,平均±標準誤差,*P < 0.05,ピアソン積率相関分析

図 47 タイプ A 行動パターン得点と左前頭前野活動の関係 以下に、タイプ A 行動パターン得点と右前頭前野活動の関係を示す。

得点の上昇とともに、ハイレゾ音においては活動が上昇する傾向にあり、MP3 音においては活動が低下することが分かった。図 43 に示すように、44 点以上のタイプ A 群においては、ハイレゾ音と MP3 音間に差異が認められている。



● ハイレゾ ◎ MP3

図 48 タイプ A 行動パターン得点と右前頭前野活動の関係

森林の MP3 音による聴覚刺激は、ハイレゾ音と比較し、

(1)被験者全体において

1)脳前頭前野活動が有意に低下すること

- (2)タイプA・B 群分類において
- 1)タイプA群においては、脳前頭前野活動が有意に低下するが、タイプB群では、有 意差が認められないこと
- 2)タイプA行動パターン得点と脳前頭前野活動の変化において、有意な相関が認められ、得点が高い程脳前頭前野活動が低下することが示された。

森林の MP3 音による聴覚刺激は、生理的・心理的リラックス効果をもたらし、その 効果は人のパーソナリティによって異なることが明らかとなった。

おわりに

本報告書においては、「フィールド実験」として、通院うつ病患者を被験者としたク リニック待合室における「木質内装壁実験」を実施し、「人工気候室内実験」として 「ハイレゾ音を用いた森林と都市の聴覚刺激実験」を実施した。

- その結果、
- (1)「フィールド実験」においては、木質内装壁の視覚刺激は、対照(白色壁)と比較して、副交感神経活動が有意に上昇し、心拍数が有意に低下することを認めた。
- (2)「人工気候室内実験」においては、森林の聴覚刺激は、都市の聴覚刺激と比較して、右前頭前野活動が有意に低下すること、交感神経活動が有意に低下すること、ならびに心拍数が有意に低下することことを認めた。
- ともに、生体が生理的にリラックスすることを示しており、世界初の知見となる。

本研究は以下のメンバーの協力の元に実施された(五十音順)。

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I期、Ⅱ期総括

I期:平成25~27年度

Ⅱ期:平成28~30年度

公益財団法人車両競技公益資金記念財団

I期、Ⅱ期総括(I期平成25~27年度、Ⅱ期平成28~30年度)

「森林浴による健康増進等に関する調査研究」は、平成 25 年~27 年度の第 I 期、 平成 28~30 年度の第 II 期に分けて実施された。

以下に、(1)本研究における「研究仮説」を示し、さらに、(2)第Ⅰ期と第Ⅱ期の 関連を述べるとともに、(3)将来展望を記す。

(1)研究仮説

森林浴という言葉は、1982年に、当時の林野庁の秋山智英長官が、日光浴や海水浴に 準じて作った言葉である。森林浴の生理的効果に関する最初の実験は、1990年3月に 日本の屋久島において宮崎によって実施された。実験開始時に測定指標として確立され た唾液中コルチゾール(ストレスホルモン)を指標として実施したが、その後、10年ほ ど、科学的データの蓄積は進まない状況が続いた。その後、2000年代に入ってから、脳 活動や自律神経活動計測法の進歩や計測機器の開発が進み、現状においては、データ蓄 積がなされつつある。2003年には「科学的裏付けのある森林浴」を意味する「森林セラ ピー」という言葉が、宮崎によって提唱されている。

一方、ここ数年、現在の都市社会における人工化がもたらすストレス性疾患が世界中 で大きな社会問題となっている。我々は、現在の人工化された都市社会において、覚醒 しすぎた状態、ストレス状態になり、病気になりやすい体になっていると考えられてい る。そのような状況下において、森林浴をはじめとする自然由来の刺激がもたらす生理 的リラックス効果に注目と期待が集まっている。2016年6月には、皇太子殿下(現天皇 陛下)・同妃殿下(現皇后陛下)が、千葉大学をご訪問になられ、森林浴等について、 講演と質疑応答を行う機会を得た。

人間は、人間となって 6~700 万年が経過するが、仮に産業革命以降を都市化、人工 化と仮定した場合、その期間は 2~300 年間に過ぎず、99.99%以上を自然環境下で過ご してきたことになる。その間、進化という過程を経て、今の人工化された社会を生きる 人間となった。遺伝子は数百年という短期間では変化できず、我々は自然環境に適応し た生体を持って今の現代社会を生きているため、必然的に、常にストレス状態にあるの である。私のこのセオリーは、ニュージーランドの研究者である MA. O'Grady and L Meinecke によって、Back-to-nature theory と命名されている。

加えて、最近の急激なコンピュータの普及はさらなるストレス状態の昂進を生み出 しており、1984年にはアメリカの臨床心理学者クレイグ・ブロードにより、「テクノ ストレス」という言葉が作られている。「森林浴」という言葉が命名されたのも1982 年であり、ここ 30年程度で第二期の人工化社会に進んだように思われる。このような 現在の人工化されたストレス社会において、我々の体が自然対応用に出来ているとい う長所を生かした森林浴効果に世界の期待と注目が集まっている。 (2)第Ⅰ期と第Ⅱ期の関連

1990年には、屋久島における森林浴実験において、唾液中ストレスホルモン(コルチ ゾール)濃度を使った生理計測が、世界で初めて実施された。しかし、それ以降、生理 指標を用いた科学的エビデンスの蓄積については、日本においても、世界においても、 長い低迷期を迎えた。

その後、平成16年になり、森林セラピー基地構想に基づいた大型実験が、継続的に 実施される体制が整い、エビデンスの蓄積が行われてきた。しかし、このエビデンスは、 20代の男女という健常者を対象とし、歩行、座観実験ともに15分~20分程度という短 時間であるという問題点を抱えていた。

そこで、平成25年度に本プロジェクトが発案された。

平成 25 年度から平成 27 年度の第 I 期においては、1) 高血圧者を含む中高年の男女 を対象として、2) 現場で実践されている 6 時間程度の森林セラピープログラム効果を 解明するためのフィールド実験が実施された。これらの実験例は、世界でも例を見ない 新規研究となった。

平成28年度から平成30年度の第II期においては、1)室内実験を中心に、2)視覚、 嗅覚、聴覚刺激に分けて、それらの単独刺激ならびに複合刺激実験をが行われ、森林浴 効果のメカニズムの解明が行われた。3)さらに、うつ病患者や脊髄損傷車椅子患者等 の強いストレス状態にある方々を対象とした実験も実施された。これらの研究も、世界 をリードする新規性の高い研究例となった。

(3) 将来展望

第 I 期の研究において、1) 中高年男女ならびに高血圧者を対象とした1日タイプ(9 時~15 時)の森林セラピープログラム実験において、高い森林セラピー効果が認めら れることが明らかとなった。第 II 期の研究においては、1) 視覚、嗅覚、聴覚刺激に分 けた室内実験において、各感覚に分けた単独刺激でもリラックス効果があること、2) 複合刺激においては、単独刺激が足された相加効果があること、3) うつ病患者や脊髄 損傷車椅子患者等の強いストレス状態にある方々においては、健常者よりも強い森林セ ラピー効果が認められることが明らかとなった。

これらの成果を踏まえた将来展望としては、1)強いストレス状態にあるうつ病患者、 ギャンブル依存症患者、車椅子患者、リハビリ患者等を対象としたフィールド実験が望 まれている。さらに、2)森林浴研究は、個人の好みが実験結果に反映される「能動的 快適性」研究であるため、大きな「個人差」が生じる。従来の科学においては、「個人 差研究」はアプローチ法がないため、避けられてきたが、今後は、積極的に「個人差研 究」に踏み込むべきである。

これらの科学的データが蓄積されることにより、個々人においては、生活の質(QOL・ Quality of life)の改善に繋がり、社会への貢献としては、予防医学的効果による医 療費削減に繋がる。

現在、「森林浴」は「Shinrin-yoku」として通用する英語となり、その健康増進効果 に世界中の関心が集まっている。日本発の「森林浴/Shinrin-yoku」を世界にアピール するべき重要な時期において、このようなプロジェクトを発案されたご慧眼に敬意を表 したい。

以下に年度毎の成果を生理指標毎に整理するとともに掲載論文リストを記した。 平成 25 年度

男性中高年高血圧者を被験者とし、10分間程度の短時間刺激と6時間程度の森林セラピープログラム効果実験を実施した。

1) 短時間·午前座観実験

①高血圧群において、リラックス時に高まる副交感神経活動が有意に上昇した。

- 2) 短時間・午後座観実験①副交感神経活動が有意に上昇した。②心拍数を有意に低下した。
- 3) 短時間・午前歩行実験①副交感神経活動が有意に上昇した。②心拍数が有意に低下した。
- 4)6時間・森林セラピープログラム実験
 ①収縮期血圧ならびに拡張期血圧が有意に低下した。
 ②血中コルチゾール濃度が有意に低下した。
 ③尿中アドレナリン濃度が有意に低下した。

平成 26 年度

女性中高年者を被験者とし、10分間程度の短時間刺激と5時間程度の森林セラピー プログラム効果実験を実施した。

1) 短時間·午前座観実験

①心拍数が有意に低下した。

2) 短時間・午後座観実験

①心拍数が有意に低下した。

- 3) 短時間・午前歩行実験
 ①副交感神経活動が有意に上昇した。
 ②心拍数が有意に低下した。
- 4)短時間・午後歩行実験①副交感神経活動が有意に上昇した。②心拍数が有意に低下した。

5)5時間・森林セラピープログラム実験

①脈拍数が有意に低下した。

②唾液中コルチゾール濃度が有意に低下した。

平成 27 年度

男性境界域高血圧または高血圧症(40~69歳)を被験者とした。11時から15時までの4時間の森林セラピープログラムによって以下の結果が得られた。

①脈拍数が有意に低下した。

②報酬系神経伝達物質である尿中ドーパミンの変化に良い影響を与えた。

③血中アディポネクチンの変化に良い影響を与えた。

平成 28 年度

以下の室内実験を実施した。

- (1) 大型ディスプレイを用いた森林視覚刺激実験
- (2) 視覚ならびに嗅覚複合刺激実験
- (3) 脊髄損傷車椅子患者に対する森林盆栽視覚刺激実験

その結果、

- (1) 大型ディスプレイを用いた森林視覚刺激実験
- ①右前頭前野の酸素化ヘモグロビン濃度が有意に低下した。
- (2) 視覚ならびに嗅覚複合刺激実験
- ①森林の嗅覚刺激ならびに複合刺激時に左右前頭前野における酸素化ヘモグロビン 濃度が有意に低下した。
- ②視覚刺激時に交感神経活動が有意に低下した。
- (3) 脊髄損傷車椅子患者に対する森林盆栽視覚刺激実験
- ①左前頭前野における酸素化ヘモグロビン濃度が有意に低下した。
- ②副交感神経活動が上昇した。

③交感神経活動が有意に低下した。

平成 29 年度

以下の室内ならびにフィールド実験を実施した。

(1) 視覚および聴覚刺激がもたらす森林浴効果の解明-室内実験から-

①左右前頭前野の酸素化ヘモグロビン濃度が、複合刺激において、有意に低下した。

- (2) うつ病患者を被験者としたビオトープがもたらす効果の解明-フィールド実験から-
 - ①交感神経活動が有意に低下した。

②副交感神経活動が有意に上昇した。

平成 30 年度

「フィールド実験」として、通院うつ病患者を被験者としたクリニック待合室にお ける「木質内装壁実験」を実施した。「人工気候室内実験」としては、「ハイレゾ音を 用いた森林と都市の聴覚刺激実験」を実施した。

(1) 木質内装壁実験

①副交感神経活動が有意に上昇した。

②心拍数が有意に低下した。

ハイレゾ音を用いた森林と都市の聴覚刺激実験

①右前頭前野活動が有意に低下した。

②交感神経活動が有意に低下した。

③心拍数が有意に低下した。

業績リスト(論文はリスト頁数参照)

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Article

Effect of Forest Walking on Autonomic Nervous System Activity in Middle-Aged Hypertensive Individuals: A Pilot Study

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Abstract: There has been increasing attention on the therapeutic effects of the forest environment. However, evidence-based research that clarifies the physiological effects of the forest environment on hypertensive individuals is lacking. This study provides scientific evidence suggesting that a brief forest walk affects autonomic nervous system activity in middle-aged hypertensive individuals. Twenty participants (58.0 \pm 10.6 years) were instructed to walk predetermined courses in forest and urban environments (as control). Course length (17-min walk), walking speed, and energy expenditure were

equal between the forest and urban environments to clarify the effects of each environment. Heart rate variability (HRV) and heart rate were used to quantify physiological responses. The modified semantic differential method and Profile of Mood States were used to determine psychological responses. The natural logarithm of the high-frequency component of HRV was significantly higher and heart rate was significantly lower when participants walked in the forest than when they walked in the urban environment. The questionnaire results indicated that, compared with the urban environment, walking in the forest increased "comfortable", "relaxed", "natural" and "vigorous" feelings and decreased "tension-anxiety," "depression," "anxiety-hostility," "fatigue" and "confusion". A brief walk in the forest elicited physiological and psychological relaxation effects on middle-aged hypertensive individuals.

Keywords: forest therapy; urban environment; walking; hypertension; middle-aged individuals; preventive medicine; heart rate variability; heart rate; semantic differential method; profile of mood state

1. Introduction

During the seven-million-year history of humans [1], they have lived in natural environments; thus, they experienced a drastic change when they began living in urban environments. Rapid urbanization and artificialization have affected the environment by increasing traffic along with air and water pollution, while decreasing the amount of available agricultural land and open spaces [2]. These environmental changes, especially climate changes, threaten human health and quality of life (QOL) [2–5]. Furthermore, the rapid development of information technology has increased what Brod describes as "technostress" [6], a modern disease of adaptation caused by unhealthy coping mechanisms for dealing with new computer technologies. When combined, these factors can severely affect humans. Several studies have reported that urban environments are stressful [7–9] and are associated with increasing mortality rates [10].

In our stressful modern age, the relaxing effects of a natural environment are very important. As our interest in improving health and QOL has increased, more attention has been focused on the role of nature in promoting human health and well-being. In particular, a great deal of attention is focused on the therapeutic effects of the forest environment or "forest therapy." Forest therapy uses the medically proven effects of walking in a forest and observing the environment to promote feelings of relaxation and improve both physical and mental health.

Many studies have demonstrated that a forest environment can have positive physiological and psychological effects [11–27]. When compared with an urban environment, viewing forest scenery or walking in forests can decrease cerebral blood flow in the prefrontal cortex [11], reduce blood pressure [12–15] and pulse rate [12–14,16,17], increase parasympathetic nerve activity [12,14–19], suppress sympathetic nerve activity [12,14,15,17–19], and decrease salivary cortisol concentrations of stress hormones [11–13,15–17]. In addition, a previous study reported that visiting a forest enhanced natural killer cell activity and improved immune function [20], and these effects continued for up to

1 month [21,22]. With regard to the psychological effects, several questionnaire-based studies reported that people who are in a forest environment experience positive feelings, which they describe as "comfortable", "soothed" and "natural" [11–14,17], as well as an improved mood and cognitive functioning [15,17–19,23–27].

Forest therapy has recently attracted attention as a preventive or alternative therapy [28,29], and its effects have been studied in elderly individuals and patients with reversible diseases. Lee and Lee [30] demonstrated that walking in a forest for 1 h improves arterial stiffness and pulmonary function in elderly women. Otsuka *et al.* [31] clarified that forest walking decreased blood glucose levels in patients with non-insulin-dependent diabetes mellitus. Other findings have indicated that cognitive behavioral therapy conducted in a forest environment was more successful in achieving depression remission than psychotherapy conducted in a hospital [32].

Several studies have demonstrated positive effects in hypertensive individuals. Mao *et al.* [33] reported that a seven-day forest-bathing trip reduces blood pressure and decreases pathological indicators of cardiovascular disease. Sung *et al.* [34] demonstrated that a frequent and eight weeks' forest therapy program based on cognitive behavioral therapy can reduce salivary cortisol levels and improve QOL in hypertensive patients. However, to the best of our knowledge, there are no evidence-based research studies that have used indices of autonomic nerve system activity to clarify the acute response of exposure to a forest environment.

Therefore, the purpose of the present study was to clarify the acute response of forest walking on autonomic nerve activity. We used heart rate variability (HRV) [35,36] and heart rate to measure autonomic responses and then compared these responses among middle-aged hypertensive individuals who walked in a forest and an urban environment.

2. Materials and Methods

2.1. Participants

Twenty Japanese men (mean age, 58.0 ± 10.6 years; mean body mass index, 23.4 ± 3.3 kg/m²) participated in the experiment. The participants' information and characteristics are shown in Table 1. Participants who were taking medication for chronic conditions such as diabetes, hyperlipidemia, and hypertension were excluded. Among these 20 participants, five had a high-normal blood pressure (systolic 130–139 mmHg or diastolic 85–89 mmHg) that was considered in the higher range of prehypertension. Of the remaining 15 participants, 10 had hypertension stage 1 (systolic 140–159 mmHg or diastolic 90–99 mmHg) and five had hypertension stage 2 (systolic 160–179 mmHg or diastolic 100–109 mmHg). Furthermore, for the classification, the values measured in the morning (8:30–8:45) of the first experimental day were used.

Before the experiment, the participants were fully informed about the study aims and procedures; and after receiving a description of the experiment, they signed an agreement to participate in the study. Consumption of alcohol and tobacco was prohibited and consumption of caffeine was controlled during the study period. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committees of the Nagano Prefectural Kiso Hospital, Japan

and of the Center for Environment, Health, and Field Sciences, Chiba University, Japan (Project identification code number: 5).

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Davamatar	Value
Parameter	(Mean ± Standard deviation)
Total sample number	20
Sex	Male
Age (years)	58.0 ± 10.6
Height (cm)	167.9 ± 6.2
Weight (kg)	66.1 ± 10.6
BMI (kg/m ²)	23.4 ± 3.3
SBP (mmHg)	151.2 ± 17.9
DBP (mmHg)	90.7 ± 5.0

Table 1. Participant	demographics	5.
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2.2. Experimental Sites

The field experiments were conducted in a coniferous forest that included many Japanese cypress trees (Akasawa Shizen Kyuyourin; Akasawa natural recreation forest) and was located in Agematsu town of Nagano Prefecture situated in central Japan (hereafter referred to as the forest area). An urban area in Ina City of Nagano Prefecture was selected as the control site (hereafter referred to as the urban area). The weather was sunny on the days of experiments. In the forest area, the average temperature was 21.4 ± 1.2 °C with an average humidity of $82.3 \pm 4.8\%$, whereas in the urban area, the average temperature was 28.1 ± 1.1 °C with an average humidity of $61.9 \pm 4.5\%$.

2.3. Physiological Indices

HRV and heart rate, which were used to quantify autonomic nervous system responses, were measured using a wearable electrocardiogram sensing system (myBeat; Union Tool, Co., Tokyo, Japan). Frequency spectra were generated using an HRV software tool (MemCalc/Win; GMS, Tokyo, Japan) [37]. For real-time HRV analysis by the maximum entropy method, interbeat (R-R) intervals were obtained continuously. In this study, two broad HRV spectral components were calculated: low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.40 Hz). The HF component is an estimate of parasympathetic nerve activity, whereas the LF/HF ratio is an estimate of sympathetic nerve activity [35,36]. To normalize HRV parameters across participants for the analysis, we transformed the values using the natural logarithm [38].

2.4. Psychological Indices

The participants answered two questionnaires to investigate psychological responses. The modified semantic differential (SD) method [39] used three pairs of adjectives on thirteen scales, including "comfortable to uncomfortable", "relaxed to awakening" and "natural to artificial". The Profile of Mood State (POMS) [40–42] scores were determined for the following six subscales:

"tension-anxiety", "depression", "anger-hostility", "fatigue", "confusion" and "vigor". We used a short version of the POMS that included 30 questions in order to decrease the participants' burden.

2.5. Experimental Design

We performed a within-subject experiment. The 20 participants were randomly assigned to two groups of 10 each that participated in the experiment over two consecutive days. On the first day, one group traveled to the forest area and the other traveled to the urban area by car (about 45 min). On the second day, the groups switched walking courses to eliminate an order effect.

The participants moved within their respective experimental site once they arrived. After resting for 10 min, the participants were instructed to walk a predetermined course. An experimenter guided the participants along the course; the duration of each walk was 17 min (Figure 1), and the two experimenters leading the courses walked at almost the same speed. The course duration and walking speed of both the experimenters were set to be the same for both the forest and urban areas. The walking course in the forest area was mostly flat, except for a small slope (3.25%) in the first 6 min of the course, whereas that of the urban area was flat. The participants walked the two courses at approximately the same time of day (10:30–11:10) to eliminate the influence of diurnal changes on physiological rhythms.

HRV and heart rate data were collected at 1-min intervals and then averaged over the entire 17-min course. We then compared these average values between sites. Energy expenditure for walking was also assessed (Lifecorder GS4; Suzuken Co., LTD., Chiba, Japan). The participants answered the two questionnaires after completing each course.



The forest area

The urban area

Figure 1. Experimental sites.

2.6. Statistical Analyses

Physiological data of 19 participants were used for analysis because of errors in data collection for one participant. We used the paired t-test to compare the mean HRV and heart rate between the two walking sites. We used the Wilcoxon signed-rank test to analyze differences the psychological indices completed after walking in each environment. All statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). In all comparisons, a *p*-value of <0.05 was considered statistically

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significant. One-sided tests were used for both comparisons because our hypothesis was that elderly hypertensive individuals would also be relaxed after walking in a forest.

3. Results

We confirmed there were no significant differences in the energy expenditure between the two environments (forest, 1.99 kcal/min; urban, 2.03 kcal/min, p > 0.05). However, the participants showed significant differences in their physiological and psychological responses for the 17-min walk in the forest and the urban areas.

Figure 2 shows the natural logarithm of HF component ln(HF), which is an estimate of parasympathetic nerve activity. In the 1-min segment analysis, most ln(HF) values were higher when participants walked in the forest than when they walked in the urban area, except during the first 4-min period (Figure 2A). The mean ln (HF) over the entire walking period was significantly higher in forest walking than in urban walking (forest, $3.9 \pm 0.2 \text{ lnms}^2$; urban, $3.5 \pm 0.2 \text{ lnms}^2$; p < 0.05, Figure 2B). In contrast, there was no significant difference between the two environments for the natural logarithm of LF/HF (ln(LF/HF)), an estimate of sympathetic nerve activity.

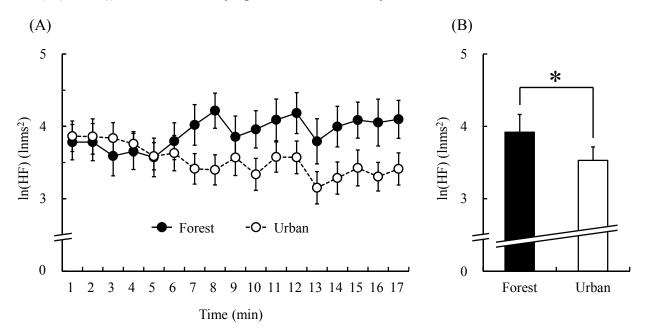


Figure 2. $\ln(\text{HF})$ value of heart rate variability during the forest and urban walk. (A) Changes in each 1-min average $\ln(\text{HF})$ value over the 17-min walk. (B) Overall mean $\ln(\text{HF})$ values. N = 19, mean \pm standard error. * p < 0.05, paired *t*-test.

Heart rate values were lower in forest walking than in urban walking, except during the first 6-min period (Figure 3A). The mean heart rate during the entire 17-min walk was significantly lower when participants walked in the forest area than when they walked in the urban area (forest, 77.1 ± 2.0 bpm; urban, 78.6 ± 1.8 bpm; p < 0.05, Figure 3B).

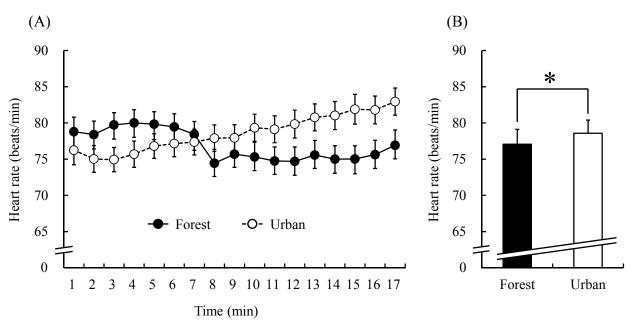


Figure 3. Heart rate during the forest and urban walk. (A) Changes in each 1-min heart rate value over the 17-min walk. (B) Overall mean heart rates. N = 19, mean \pm standard error. * p < 0.05, paired *t*-test.

Our analysis of the participants' responses to the two questionnaires, the SD method and the POMS scores, revealed differences in psychological responses between the two environments. Participants felt more "comfortable", "relaxed" and "natural" when they walked in the forest area than in the urban area (p < 0.01, Figure 4). We also observed differences in the POMS test in which scores for the negative subscales of "tension–anxiety", "depression", "anger-hostility", "fatigue" and "confusion" were significantly lower after walking in the forest area than after walking in the urban area (p < 0.05, Figure 5). Conversely, the positive mood state "vigor" was significantly higher after walking in the forest area than after solution.

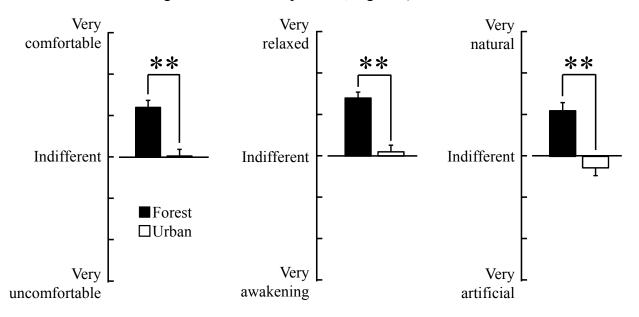


Figure 4. Comparison of "comfortable," "relaxed," and "natural" feeling scores between the two environments. N = 20, mean \pm standard error. ****** p < 0.01, Wilcoxon signed-rank test.

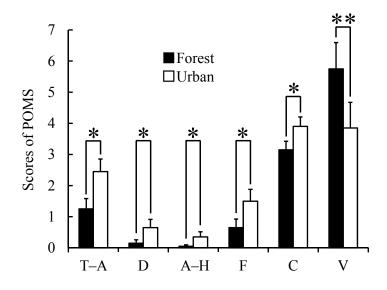


Figure 5. Comparison of Profile of Mood State (POMS) scores between the two environments. T–A: tension–anxiety; D: depression; A–H: anger–hostility; F: fatigue; C: confusion; V: vigor. N = 20, mean \pm standard error. * p < 0.05, ** p < 0.01, Wilcoxon signed-rank test.

4. Discussion

A short walk in a forest can have significant physiological and psychological effects on middle-aged hypertensive individuals. Compared with walking in the urban environment, walking in the forest environment significantly increased parasympathetic nerve activity and significantly decreased heart rate. These results are consistent with those from previous studies that examined physiological responses to a forest environment in young adults [12,14–19]. HRV responses are often detected during relaxed states such as during rest [35], a massage [43,44], or after performing yoga [45]. Therefore, we concluded that participants who walked in the forest were in a physiologically relaxed state.

On the other hand, we observed the reverse in our analysis of ln(HF) and heart rate for the 1-min segments. We do not know the exact reason for this difference; however, because heart rate increases during walking and running, especially uphill [46–48], we suppose these physiological responses resulted from the small slope at the beginning of the forest area course. The slope was characterized by a forest environment. We believe that if this feature is used well, it will be of great merit to forest therapy programs.

In the questionnaires, the participants reported that they felt more "comfortable", "relaxed" and "natural" after walking in the forest. In addition, negative emotions such as "tension-anxiety," "depression", "anger-hostility", "fatigue" and "confusion" as well as the positive emotion of "vigor" improved significantly after walking in the forest. Our findings of the psychological benefits of walking in a forest are partly consistent with previous findings [11–15,18,23]. In the modern age, the importance of mental health has increased [49]. The psychological benefits of a forest environment may play a very important role in improving mental stress.

Furthermore, physical condition such as the average temperature (forest: 21.4 ± 1.2 °C, urban: 28.1 ± 1.1 °C) and humidity (forest: $82.3 \pm 4.8\%$, urban: $61.9 \pm 4.5\%$) was significantly different

(p < 0.01 by unpaired t-test). Park *et al.* [23] examined the relationship between psychological responses to forest and urban areas and the physical variables of these environments. As a result, the psychological responses to physical environments were also significantly related to air temperature, relative humidity, radiant heat, wind velocity, PMV, and PPD. It is considered that different physical condition is one of the reasons for differences in physiological and psychological responses in the present results.

Walking is a simple, accessible, and cost-effective method to improve physical health, and this has been clarified in previous studies [50,51]. Iwane *et al.* [50] reported that walking at least 10,000 steps per day can lower blood pressure and suppress sympathetic nerve activity in hypertensive patients. Williams and Thompson [51] demonstrated that equivalent energy expenditures in walking and running could produce similar risk reductions for hypertension, hypercholesterolemia, and diabetes mellitus. However, it is not yet elucidated whether such effects can be attributed to differences in the walking environment.

The present findings suggest that these effects can differ with the environment. The present findings also clearly demonstrate that in middle-aged men, a brief walk in the forest was associated with relaxing physiological and psychological effects. However, this study had a few limitations. To generalize the findings, it is necessary to consider the following: First, these results cannot be extrapolated to the female population and people of different age groups. Further studies on a large sample including various subject groups are required. Second, the present study only used HRV and heart rate. For the overall discussion, future studies should be assessed to determine the effects of forest environment using other physiological indices, such as brain activity, autonomic nervous activity and endocrine activity.

5. Conclusions

Regarding the physiological and psychological effects of a brief walk in the forest environment for middle-aged individuals with hypertension, our study findings revealed the following: (1) a significant increase in parasympathetic nerve activity; (2) a significant decrease in heart rate; (3) a significant increase in "comfortable," "relaxed," and "natural" feelings assessed by the modified SD method combined with significant improvements in "tension-anxiety", "depression", "anger-hostility", "fatigue", "confusion" and "vigor" assessed by the POMS. In conclusion, walking in a forest induced physiological and psychological relaxation.

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Author Contributions

Chorong Song contributed to the experimental design, data acquisition, statistical analysis, interpretation of results, and manuscript preparation. Harumi Ikei contributed to the experimental design, data acquisition, statistical analysis, and interpretation of results. Maiko Kobayashi conducted data acquisition. Takashi Miura and Masao Taue contributed to preparing the experimental sites and

cooperated with data acquisition. Takahide Kagawa and Qing Li participated in data acquisition and contributed to the interpretation of results. Shigeyoshi Kumeda and Michiko Imai conceived the study and participated in the interpretation of results. Yoshifumi Miyazaki conceived and designed the study, contributed to the interpretation of results, and manuscript preparation. All authors have read and approved the final version submitted for publication.

Conflicts of Interest

The authors declare no conflict of interest.

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Effects of viewing forest landscape on middle-aged hypertensive men

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ABSTRACT

With increasing attention on the health benefits of a forest environment, evidence-based research is required. This study aims to provide scientific evidence concerning the physiological and psychological effects of exposure to the forest environment on middle-aged hypertensive men. Twenty participants $(58.0 \pm 10.6 \text{ years})$ were instructed to sit on chairs and view the landscapes of forest and urban (as control) environments for 10 min. Heart rate variability (HRV) and heart rate were used to quantify physiological responses. The modified semantic differential method was used to determine psychological responses. Consequently, the high-frequency component of HRV, a marker of parasympathetic nervous activity that is enhanced in relaxing situations, was significantly higher and heart rate was significantly lower in participants viewing the forest area than in those viewing the urban area. The questionnaire results indicated that viewing the forest environment increased "comfortable," "relaxed," and "natural" feelings than viewing the urban environment. In conclusion, viewing forest landscape produces physiological and psychological relaxation effects on middle-aged hypertensive men.

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1. Introduction

In recent years, there has been considerable and increased attention in using the forest environment as a place for recreation and health promotion. This approach was called "Shinrin-yoku" that means "taking in the forest atmosphere" (Selhub and Logan, 2012). It suggests that "forest bathing," which is a health promotion method and uses proven effects of a forest environment, such as relaxation, can improve the health of the body and mind. In accordance with the accumulation of data, the idea of "forest ther-

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Previous studies targeting healthy young adults have demonstrated that time spent in a forest environment can decrease cerebral blood flow in the prefrontal cortex (Park et al., 2007) decrease blood pressure (Tsunetsugu et al., 2007; Lee et al., 2009; Park et al., 2009; Park et al., 2010), reduce pulse rate (Tsunetsugu et al., 2007; Park et al., 2008; Lee et al., 2009; Park et al., 2009; Lee et al., 2011), and increase parasympathetic nervous activity that is enhanced in relaxing situations (Tsunetsugu et al., 2007; Park et al., 2008; Park et al., 2009; Park et al., 2010; Lee et al., 2011; Tsunetsugu et al., 2013; Lee et al., 2014). Sympathetic nervous activity that is enhanced in stressful situations is suppressed (Tsunetsugu et al., 2007; Park et al., 2009; Park et al., 2010; Lee et al., 2011; Tsunetsugu et al., 2013; Lee et al., 2014). In addition, the levels of salivary cortisol, a stress hormone, decrease (Miyazaki and Motohashi, 1996; Tsunetsugu et al., 2007; Park et al., 2007; Park et al., 2008; Lee et al., 2009; Park et al., 2010; Lee et al., 2011; Tsunetsugu et al., 2013). In other studies, natural killer (NK) cell activity was enhanced and immune function was improved; these effects lasted for 30 days (Li et al., 2007; Li et al., 2008a, 2008b).

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Abbreviations: HRV, heart rate variability; ICC, intraclass correlation coefficient; NK, natural killer; LF, low frequency; HF, high frequency; SD, semantic differential.

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apy" has been proposed. It means evidence-based "forest bathing (shinrin-yoku)" and aims to achieve a preventive medical effect by inducing physiological relaxation and immune system recovery.

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From the psychological aspect, restorative effects related to psychological stressors or mental fatigue and improved mood states and cognitive function (Miyazaki and Motohashi, 1996; Li et al., 2007; Morita et al., 2007; Shin et al., 2010; Park et al., 2011; Shin et al., 2011) have been reported.

Studies targeting elderly individuals and patients with reversible diseases have also been reported. Walking in a forest environment can improve arterial stiffness and pulmonary function in elderly women (Lee and Lee, 2014). Furthermore, it can decrease blood glucose levels in patients with non-insulindependent diabetes mellitus (Ohtsuka et al., 1998), provide the subjective perception of having less days of pain and insomnia and more days of wellness in patients with fibromyalgia (López-Pousa et al., 2015), and enhance NK cell activation leading to the production of two anticancer molecules in breast cancer patients (Kim et al., 2015). Other findings have indicated that cognitive behavioral therapy conducted in a forest environment was more successful in achieving depression remission than psychotherapy conducted in a hospital (Kim et al., 2009).

Several studies have demonstrated the beneficial effects of forest therapy in hypertension. Forest therapy programs such as walking, guided activity, or educational sessions can reduce blood pressure (Mao et al., 2012; Ochiai et al., 2015), urinary adrenaline concentration (Ochiai et al., 2015), and serum and salivary cortisol levels (Sung et al., 2012; Ochiai et al., 2015) in hypertensive individuals. Hypertension is a critical public health challenge worldwide, and the prevention, detection, treatment, and control of this condition have been emphasized (Kearney et al., 2005). Forest therapy is expected to play a key role in this respect. A previous study examined the effects of walking in a forest environment on middleaged hypertensive men (Song et al., 2015a). Walking in a forest environment can enhance parasympathetic nervous activity and decrease heart rate in hypertensive individuals compared with walking on the city streets (Song et al., 2015a). However, these findings included not only the impact of forest environment on humans but also incorporated an element of exercise; thus, one must be careful not to over-interpret the health-giving properties of a forest environment alone. Evidence-based research concerning only the influence of exposure to a forest environment while remaining sedentary is lacking. To the best of our knowledge, there are no studies that have examined the physiological and psychological effects of viewing a forest environment in a seated position in hypertensive individuals.

The present study aimed to clarify the effects of viewing forest landscape on the autonomic nervous activity of middle-aged hypertensive men who remained sedentary while viewing the landscape.

2. Materials and methods

2.1. Participants

Japanese men between the ages of 40 and 75 years and whose blood pressures were above the upper boundary of normal (120/80 mmHg) were recruited. Researchers contacted applicants face-to-face before the start of the study, and those who were taking daily medication for chronic conditions, such as diabetes, hyperlipidemia, and hypertension, were excluded. In total, 20 Japanese men aged 40–72 years (mean age, 58.0 ± 10.6 years; Table 1) participated. Among them, eight participants lived in cities with more than 50,000 residents, nine lived in towns with more than 8000 residents, and three lived in villages with less than 8,000 residents.

Of these 20 participants, five had a high-normal blood pressure (systolic, 130–139 mmHg or diastolic, 85–89 mmHg) that was considered to be on the higher range of pre-hypertension. Of the remaining 15 participants, 10 had hypertension stage

Table 1
Participant demographics.

1 01	
Parameters	Value
	(Mean ± Standard deviation)
Total sample number	20
Sex	Male
Age (years)	58.0 ± 10.6
Height (cm)	167.9 ± 6.2
Weight (kg)	66.1 ± 10.6
BMI (kg/m ²)	23.4 ± 3.3
SBP (mmHg)	151.2 ± 17.9
DBP (mmHg)	90.7 ± 5.0

1 (systolic, 140–159 mmHg or diastolic, 90–99 mmHg) and five had hypertension stage 2 (systolic, 160–179 mmHg or diastolic, 100–109 mmHg). For classification, the values measured in the morning (8:30–8:45) of the first experimental day at the Nagano Prefectural Kiso Hospital were used. Furthermore, systolic and diastolic blood pressures were measured according to the oscillometric method using a digital blood pressure monitor (HEM1020; Omron Corp., Kyoto, Japan).

At the beginning of the experiment, the participants were informed about the aims and procedures of the study. After receiving a description of the experiment, they signed an agreement to participate in the study. During the study period, the consumption of alcohol, caffeine, and tobacco was prohibited. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committees of the Nagano Prefectural Kiso Hospital, Japan and of the Center for Environment, Health and Field Sciences, Chiba University, Japan (Project identification code number: 5).

2.2. Experimental sites

The field experiment was conducted in a natural coniferous forest that included many Japanese cypress trees (Akasawa natural recreation forest) and was located in Agematsu town of Nagano Prefecture, which is situated in central Japan (hereafter referred to as the forest area). In Japan, Japanese cypress is a well-known and common tree, and coniferous forests are typical. The urban environment is used as a control, which is a common exposure in everyday life. The urban areas were downtown near the Japan Railway station (hereafter referred to as the urban area).

The weather was sunny on the days of experiments. During viewing of the forest area, the average temperature was $24.3 \degree C \pm 0.1 \degree C$ with an average humidity of $70.5\% \pm 0.9\%$, whereas in the urban area, the average temperature was $29.9 \degree C \pm 0.1 \degree C$ with an average humidity of $52.0\% \pm 0.8\%$.

2.3. Experimental design

The 20 participants were randomly assigned to two groups of 10 that participated in the experiment over 2 consecutive days. On the first day (September 14), one group moved to the forest area and the other moved to the urban area by car (an approximately 45-min journey). On the second day (September 15), the groups switched experimental areas to eliminate an order effect.

The participants moved within their respective experimental site. After arriving at the site, participants were instructed to sit on a chair. After resting for 5 min, they viewed each landscape for a period of 10 min in the afternoon (Fig. 1). Conversation among participants was prohibited. Furthermore, the participants viewed the two areas at approximately the same time of day to eliminate the influence of diurnal changes on physiological rhythms.

After viewing, participants answered the questionnaires.



Fig. 1. Scenery as viewed in the forest and urban areas.

2.4. Physiological indices

Heart rate variability (HRV) and heart rate were measured to assess autonomic nervous activity. HRV and heart rate were measured using an electrocardiogram sensing system (myBeat; Union Tool Co., Tokyo, Japan). Frequency spectra were generated using a HRV software tool (MemCalc/Win; GMS, Tokyo, Japan). For real-time HRV analysis using the maximum entropy method, interbeat (R–R) intervals were continuously obtained. In this study, the following two broad HRV spectral components were calculated: low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.40 Hz). The HF component is an estimate of the parasympathetic nervous activity, whereas the LF/HF ratio is an estimate of the sympathetic nervous activity (Pagani et al., 1986; Task force of the European society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Several studies have investigated the reliability of HRV measurement by calculating the intraclass correlation coefficient (ICC). Kowalewski and Urban (2004) reported the short- and long-term reproducibility of HRV parameters according to body position (supine or standing). The ICCs of the components HF and LF/HF were 0.71–0.89 and 0.54–0.85, respectively. Kobayashi (2009) reported that the ICCs of logarithmic transformed components (InHF and InLF) were 0.71–0.88 regardless of whether paced breathing was applied. Bertsch et al. (2012) demonstrated a value of 0.70–0.73 for the ICC of the parasympathetic indicator of HRV. Most of these studies concluded that the reliability of HRV measurement is good or excellent.

HRV and heart rate data were collected at 1-min intervals and averaged over the entire 10-min period. We then compared these average values between sites.

2.5. Psychological indices

The modified semantic differential (SD) method (Osgood et al., 1957) was used to evaluate the psychological responses of the participants. This method tests the subjective spatial impressions of participants through a questionnaire with three pairs of opposing adjectives, each of which is evaluated on 13 scales, including "comfortable to uncomfortable," "relaxed to awakening," and "natural to artificial." The higher the score for each, the better the emotional condition.

2.5.1. Statistical analyses

Physiological data of 19 participants were used for analysis because of errors in data collection for one participant. We used the paired *t*-test to compare the mean HRV and heart rate between the two sites. Wilcoxon signed-rank test was used to analyze differences between the psychological indices. All statistical analyses were performed using SPSS, version 20.0 (IBM Corp., Armonk, NY, USA). In all comparisons, p < 0.05 was considered statistically significant. One-sided tests were used for both comparisons because our hypothesis was that middle-aged hypertensive men would be relaxed by viewing a forest environment than by viewing an urban environment.

3. Results

The participants showed significant differences in their physiological and psychological responses for the 10-min viewing of forest and urban areas.

Fig. 2 shows the HF component, which is an estimate of parasympathetic nervous activity, to be enhanced in relaxing situations. In the 1-min segment analysis, all HF values were higher when participants viewed the forest area than when they viewed the urban area (Fig. 2a). The mean HF over the entire viewing period was significantly higher in the forest area than in the urban area (forest, $142.4 \pm 28.1 \text{ ms}^2$; urban, $97.0 \pm 17.4 \text{ ms}^2$; p < 0.05, Fig. 2b). However, there was no significant difference between the two environments for LF/HF, an estimate of sympathetic nervous activity that is enhanced in stressful situations (forest area, 5.8 ± 1.0 ; urban area, 6.0 ± 1.1 ; p > 0.05).

Heart rates were lower in the forest area than in the urban area in all 10-min periods (Fig. 3a). The mean heart rate was significantly lower when participants viewed the forest area than when they viewed the urban area (forest, 69.8 ± 1.6 bpm; urban, 72.4 ± 1.3 bpm; p < 0.01, Fig. 3b).

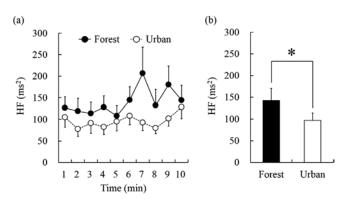
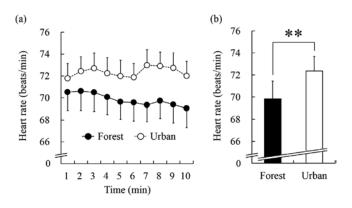
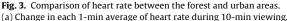


Fig. 2. Comparison of HF value of heart rate variability between the forest and urban areas.

(a) Change in each 1-min average of HF value during 10-min viewing.
 (b) Overall mean HF values.

N = 19, mean \pm standard error. *P < 0.05, determined using the paired *t*-test (one-sided).





(b) Overall mean heart rates.

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N = 19, mean \pm standard error. **P < 0.01, determined using the paired *t*-test (one-sided).

Our analysis of the participant responses to the SD method revealed differences in psychological responses between the two environments. Participants felt more "comfortable," "relaxed," and "natural" when they viewed the forest area than when they viewed the urban area (p < 0.01, Fig. 4).

4. Discussion

Viewing forest landscape can have significant physiological and psychological relaxation effects on middle-aged hypertensive men. Compared with the urban environment, a view of forest environment landscapes for 10 min significantly increased parasympathetic nervous activity and decreased heart rate. In the questionnaires, participants reported that they felt more "comfortable," "relaxed," and "natural" after viewing the forest. Our findings are consistent with those of previous studies that examined physiological and psychological responses to a forest environment (Tsunetsugu et al., 2007; Park et al., 2008; Park et al., 2009; Park et al., 2010; Park et al., 2011; Lee et al., 2011; Tsunetsugu et al., 2013; Lee et al., 2014). These results may support the possibility that just being in a forest environment for a short period of time can be relaxing both physically and mentally.

In a previous study, we examined the effects of walking in a forest area compared with walking in an urban area (Song et al., 2015a) using a similar experimental design and the same locations and participants as in this study. In the previous study, partici-

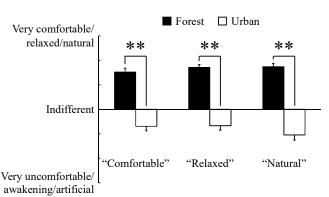


Fig. 4. Comparison of subjective scoring for "comfortable," "relaxed," and "natural" feelings between the two environments according to the semantic differential method.

N=20, mean \pm standard error. **P<0.01, determined using the Wilcoxon signed-rank test (one-sided).

pants walked in both forest and urban areas for 17 min. The HF value was $107.1 \pm 31.2 \text{ ms}^2$ during walking in the forest area and $56.0 \pm 14.3 \text{ ms}^2$ during walking in an urban area. In present study, the HF value was $142.4 \pm 28.1 \text{ ms}^2$ during viewing a forest area and $97.0 \pm 17.4 \, \text{ms}^2$ during viewing an urban area. HF values during both viewing and walking were higher in the forest area than in the urban area. In addition, they were higher when viewing landscape than when walking. Heart rate was 77.1 ± 2.0 bpm when walking in the forest area and 78.6 ± 1.8 bpm when walking in the urban area. In the present study, the heart rate was 69.8 ± 1.6 bpm while viewing the forest area and 72.4 ± 1.3 bpm while viewing the urban area. Heart rate while viewing and walking was lower in the forest area than in the urban area. In addition, it was lower while viewing landscape than while walking. The present study shows that the physiological effects of a forest environment alone without incorporating an element of exercise.

Regarding the differences in physiological effect in the forest environment, it may be influenced by various physical factors, such as temperature, humidity, atmospheric pressure, and wind speed, as well as by differences in the stimuli that affect the five senses in a forest environment. Lee et al. (2009) reported significant differences in temperature and humidity between forest and urban environments and discussed the relationship between temperature and human physiological responses. The heart rate is known to decrease at a low ambient temperature (Ishibashi and Yasukouchi, 1999); however, studies on the effect of ambient temperature changes on HRV are lacking. In the present study, the difference in average temperature and humidity between the two environments was 5.6 °C and 18.5%, respectively. Future studies should evaluate the physiological effects with comparable temperature and humidity.

The reasons why these effects can be achieved in a forest environment have not been identified. However, some theories exist. Ulrich et al. (1991) and Ulrich (1983) developed a "psychoevolutionary theory," which suggested that during evolution, there were definite advantages in acquiring a capacity for restoration in response to certain unthreatening natural conditions. Therefore, modern civilization may have an inherent preparedness to quickly and readily acquire restorative responses to many unthreatening natural environments.

According to Kaplan's (1995) "Attention restoration theory," an environment that possesses a restorative effect requires the following four properties: being distinct from the daily environment either physically or conceptually (being away), containing elements that effortlessly drive attention (fascination), having scope and coherence that allows one to remain engaged (extent), and fitting with and supporting what one wants or is inclined to do (compatibility). Consequently, Kaplan argued that the natural environment satisfies these elements.

Miyazaki et al. (2011) promulgated a "back to nature" theory (O'Grady and Meinecke, 2015). Humans have spent more than 99.99% of their evolutionary history in the natural environment; thus, the human body is made to adapt to nature. Because physiological functions have adapted to the natural environment, we are unable to adjust to rapid environmental changes and may feel stressed. Thus, when we are exposed to the natural environment, our bodies revert to how they should be. Recently, Song et al. (2015b) clarified that a physiological adjustment effect moved close to an appropriate level. Participants with high initial blood pressure and pulse rate showed a decrease in these values after walking in a forest environment, whereas those with low initial values showed an increase. However, there was no physiological adjustment effect observed in those walking in an urban environment. Thus, it is clear that these effects are specific to a forest environment. These results support the "back to nature" theory.

Regarding the effects of exposure to the forest environment for 10 min on middle-aged men with hypertension, our study findings revealed the following: (1) a significant increase in parasympathetic nervous activity, (2) a significant decrease in heart rate, and (3) a significant increase in feeling "comfortable," "relaxed," and "natural" assessed by the modified SD method. In conclusion, exposure to a forest environment induced physiological and psychological relaxation.

Currently, most people live in an urban, artificial environment and are constantly exposed to stressors through the five senses (Craig, 1984; Herbert and Cohen, 1993; Patz et al., 2005; Ge'mes et al., 2008; Lederbogen et al., 2011; McKenzie et al., 2013). Therefore, these physiological and psychological benefits of the forest environment are significant, and the forest environment is expected to play a very important role in shaping health promotion in the future.

However, this study had several limitations. To generalize the findings, it is necessary to consider the following. First, these results cannot be extrapolated to the female population and people of different age groups. Further studies on a large sample, including various participant groups, are required. Second, this study only used HRV and heart rate as variables for analysis. For the overall discussion, future studies should determine the effects of the forest environment using other physiological indices, such as brain and endocrine activities.

5. Conclusions

Regarding the effects of viewing forest landscape for a short period of time on middle-aged men with hypertension, our study findings revealed the following: (1) a significant increase in parasympathetic nervous activity, (2) a significant decrease in heart rate, and (3) a significant increase in feeling "comfortable," "relaxed," and "natural" assessed by the modified SD method. In conclusion, exposure to a forest environment induced physiological and psychological relaxation.

Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Chorong Song contributed to the experimental design, data acquisition, statistical analysis, interpretation of results, and manuscript preparation. Harumi Ikei contributed to the experimental design, data acquisition, statistical analysis, and interpretation of results. Maiko Kobayashi conducted data acquisition. Takashi Miura contributed to preparing the experimental sites and cooperated with data acquisition. Qing Li and Takahide Kagawa participated in data acquisition and contributed to the interpretation of results. Shigeyoshi Kumeda and Michiko Imai conceived the study and participated in the interpretation of results. Yoshifumi Miyazaki conceived and designed the study and contributed to the interpretation of results and manuscript preparation. All authors have read and approved the final version submitted for publication.

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Communication

Physiological and Psychological Effects of Forest Therapy on Middle-Aged Males with High-Normal Blood Pressure

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Abstract: Time spent walking and relaxing in a forest environment ("forest bathing" or "forest therapy") has well demonstrated anti-stress effects in healthy adults, but benefits for ill or at-risk populations have not been reported. The present study assessed the physiological and psychological effects of forest therapy (relaxation and stress management activity in the forest) on middle-aged males with high-normal blood pressure. Blood pressure and several physiological and psychological indices of stress were measured the day before and approximately 2 h following forest therapy. Both pre- and post-treatment measures were conducted at the same time of day to avoid circadian influences. Systolic and diastolic blood pressure (BP), urinary adrenaline, and serum cortisol were all significantly lower than baseline following forest therapy (p < 0.05). Subjects reported feeling significantly more "relaxed" and "natural" according to the Semantic Differential (SD) method. Profile of Mood State (POMS) negative mood subscale scores for "tension-anxiety," "confusion," and "anger-hostility," as well as the Total Mood Disturbance (TMD) score were significantly lower following forest therapy. These results highlight that forest is a promising treatment strategy to reduce blood pressure into the optimal range and possibly prevent progression to clinical hypertension in middle-aged males with high-normal blood pressure.

Keywords: forest therapy; high-normal blood pressure; adrenaline; cortisol; preventive medicine; Semantic Differential method; Profile of Mood State

1. Introduction

While technology and modern city life offer unparalleled economic opportunities, conveniences, and comforts, urban environments are also stressful [1,2], which may contribute to chronic health problems. Many urban dwellers are thus looking for convenient methods of stress relief. Of these, the relaxing effects of natural environments are increasingly recognized as an effective counter to urban stress. The term "Shinrin-yoku" (taking in the atmosphere of the forest or literally "forest bathing") was coined by the Japanese Ministry of Agriculture, Forestry and Fisheries to describe the positive effects of brief sojourns in natural environments to improve general health [3]. In later years, the term "Shinrin-yoku" developed into "Forest Therapy," which uses the medically proven effects of walking and observing in a forest. Indeed, "Forest Therapy" is increasingly recognized as a relaxation and stress management activity with demonstrated clinical efficacy [4].

A variety of physiological indices show that humans are more relaxed in forested environments [3–8]. For example, a forest environment lowers blood pressure and pulse rate in humans [5–7]. Forest walking also suppresses sympathetic activity and increases parasympathetic activity [6,7] and reduces the levels of cortisol and cerebral blood flow in the prefrontal cortex [3]. It was also shown that a forest bathing trip can increase human natural killer (NK) cell activity and improve immunity in both males and females, and these effects were proved to last for at least 7 days [8]. In addition, psychological studies have demonstrated the benefits of forest environments on subjective measures of stress, cognitive function, and mood [5,6]. Park *et al.* reported the relaxation and stress management effects of forest environments by several questionnaire-based studies [9] as well as improved mood and cognitive function [10].

In psychological tests of young adult males, forest bathing significantly increased positive feeling scores and reduced negative feeling scores compared with urban stimuli [5,6,10].

However, previous studies have only investigated the physiological and psychological responses to forest bathing in healthy young adults, while such effects may be even more beneficial to middle-age subjects in the early stages of age-related diseases such as hypertension. Moreover, it is generally accepted that effects of treatment on blood pressure may vary between healthy normotensives and subjects with higher blood pressure, so studies on the latter population may be of greater clinical relevance. Therefore, the aim of this study was to measure the physiological and psychological effects of forest therapy on middle-aged males with high-normal blood pressure.

2. Materials and Methods

2.1. Participants

Nine Japanese males ranging in age from 40 to 72 years (56 ± 13.0 ; mean \pm standard deviation) participated in this experiment. Potential participants who were taking medication for chronic conditions such as diabetes, hyperlipidemia, and hypertension were excluded. All participants had high-normal blood pressure (systolic 130–139 mmHg or diastolic 85–89 mmHg) as measured at Nagano Prefecture Kiso Hospital. Systolic blood pressure ranged from 124.5 to 137.5 mmHg (131.8 \pm 4.1 mmHg) and diastolic blood pressure from 65.7 to 86.7 mmHg (77.3 \pm 7.1 mmHg).

At the beginning of the experiment, subjects gathered in a waiting room at Nagano Prefecture Kiso Hospital and were fully informed about the study aims and procedures involved. After receiving a description of the experiment, the subjects all signed an agreement to participate. To control for the effect of alcohol, subjects did not consume alcohol during the entire study period. This study was approved by the Ethics Committee of Nagano Prefecture Kiso Hospital and the Center for Environment, Health and Field Sciences, Chiba University, Japan, on 19 August 2013 and performed according to the Declaration of Helsinki (1975, revised in 2008).

2.2. Experimental Sites

The forest therapy phase was conducted in Akasawa Shizen Kyuyourin (Akasawa Natural Recreation Forest), Agematsu, Nagano Prefecture (situated in central Japan) on 7 September 2013. Distance from the waiting room at Nagano Prefecture Kiso Hospital to the forest was 21.6 km, and it took 52 min to drive by car. The weather was cloudy, with a temperature of 21.5 °C (19.1 °C–25.0 °C) and humidity of 80.4% (62%–92%) on the day of forest therapy.

2.3. Physiological Indices

Systolic and diastolic blood pressure readings were obtained from the right arm using a portable digital sphygmomanometer (HEM-1020, Omron, Kyoto, Japan). Urine and blood samples were also obtained for the measurement of adrenaline, creatinine, and cortisol, respectively. All procedures were performed between 15:14 and 15:35 on the day before and a few hours after forest therapy to control for circadian effects. Participants were not allowed to talk each other during the measurement.

2.4. Psychological Indices

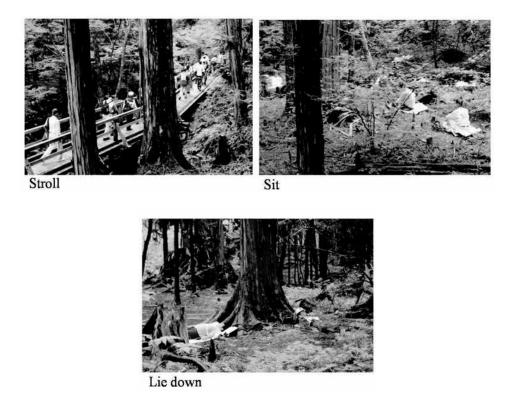
The Semantic Differential (SD) method, Profile of Mood State (POMS) subscale scores, and combined POMS Total Mood Disturbance (TMD) score were used to evaluate psychological responses to forest therapy. These questionnaires were completed by participants on the day before and soon after the experiment between 15:05 and 15:35. The SD method uses three pairs of adjectives anchoring seven-point scales: "comfortable to uncomfortable," "relaxed to awakening," and "natural to artificial" [11]. The POMS scores were determined for the following six subscales: "tension-anxiety (T-A)," "confusion (C)," "anger-hostility (A-H)," "depression (D)," "fatigue (F)," and "vigor (V)." A short form of the POMS with 30 questions was used to decrease the burden on the subjects [12]. The TMD score was calculated by combining T-A + C + A-H + D + F – V. A high TMD score indicates an unfavorable psychological state.

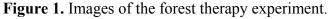
2.5. Experimental Design

Participants spent the previous night in their respective homes. In the morning of the forest therapy day, participants gathered in the same meeting room at 9:00 a.m. and participated in the forest therapy program as a group with a guide. They were not allowed to communicate with each other during forest therapy, except during lunch time and designated rest periods, and they were not permitted to carry cell phones. Participants walked around their assigned area and then sat and lay on their backs in the forest on waterproof sheets laid on the ground; this program, comprising multiple actions, was performed for 4 h and 35 min (Table 1, Figure 1).

Time	Event	Calorie Consumption (Kcal/min)
10:30-11:08	Stroll (Forest)	0.92
11:09-11:20	Sit (Forest)	0
11:21-11:26	Stroll (Forest)	0.85
11:27-11:31	Deep breathing (Forest)	0.02
11:32-11.39	Stroll (Forest)	0.71
11:40-11:49	Lie down (Forest)	0
11:50-12:17	Stroll (Forest)	1.72
12:18-13:16	Lunch and rest (Resting room)	0.12
13:17-13:30	Stroll (Forest)	0.38
13:31-13:53	Ride on the "Forest train" (Forest)	0.04
13:54-13:58	Stroll (Forest)	0.64
13:59-14:16	Stroll (Indoor pavilion)	0.31
14:17-14:32	Stroll (Forest)	0.17
14:33-15:05	Rest (Resting room)	0.05

Table 1. Time schedules of and calorie consumption during various activities of forest therapy.





Energy expenditure for the activity was assessed (Lifecorder GS4; Suzuken Co., Ltd., Chiba, Japan). Tobacco and all drinks (except mineral water) were prohibited during forest therapy. They had the same lunch, which was made at lunch time from local ingredients. The subjects walked around their assigned areas and then sat and lay on their backs for 4 h and 45 min. Subjects then returned to a waiting room and completed the post-treatment measurements and questionnaires. These results were then compared with the data obtained on the day before.

2.6. Statistical Analysis

We used paired sample t-tests to compare physiological indices and the Wilcoxon signed-rank test to compare psychological test results before and after forest bathing. All statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Data are expressed as mean \pm standard error (mean \pm SE). For all cases, p < 0.05 (one sided) was considered statistically significant.

3. Results

Both systolic and diastolic blood pressure were significantly lower after forest therapy (systolic blood pressure: before, 140.1 mmHg, after, 123.9 mmHg; diastolic blood pressure: before, 84.4 mmHg, after, 76.6 mmHg; p < 0.01) in middle-aged males with high-normal blood pressure (Figure 2). Similarly, both urinary adrenaline (with urinary creatinine correction) (before, 13.1 µg/g creatinine; after, 11.0 µg/g creatinine; p < 0.05) (Figure 3) and serum cortisol (before, 7.4 µg/dL; after, 4.9 µg/dL; p < 0.01) (Figure 4) were significantly lower after forest therapy.

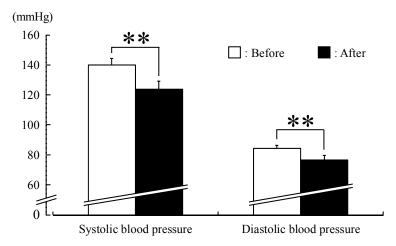


Figure 2. Effect of forest therapy on systolic and diastolic blood pressures in middle-aged males with high-normal blood pressure. N = 9, mean \pm standard error. ** p < 0.01, paired *t*-test.

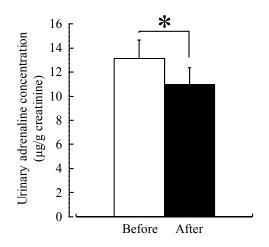


Figure 3. Effect of forest therapy on urinary adrenaline levels. N = 9, mean \pm standard error. * p < 0.05, paired *t*-test.

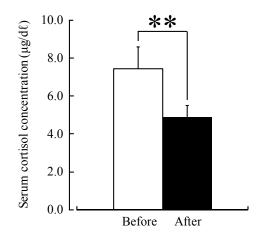


Figure 4. Effect of forest therapy on serum cortisol levels. N = 9, mean \pm standard error. ****** p < 0.01, paired *t*-test.

Significantly higher semantic differential (SD) scores were observed for the adjectives "relaxed" (p < 0.01) and "natural" (p < 0.05) after forest therapy as compared with baseline (Figure 5). Finally, a significant elevation of mood was detected on the POMS test (Figure 6), with scores for the negative subscales "tension-anxiety" (p < 0.01), "confusion," and "anger-hostility" and the TMD significantly lower after forest therapy (p < 0.05).

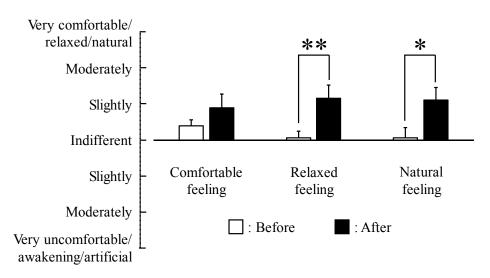


Figure 5. Semantic Differential (SD) method scores before and after forest therapy. Changes in the subjective feelings "comfortable," "relaxed," and "natural." N = 9, mean \pm standard error. ** p < 0.01, * p < 0.05, Wilcoxon signed-rank test.

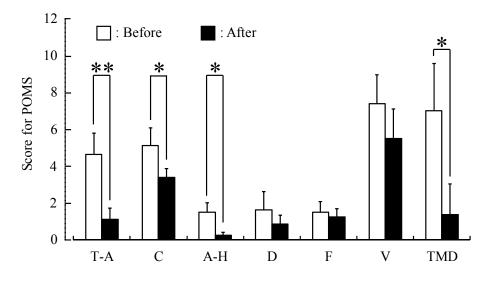


Figure 6. Subjective Profile of Mood State (POMS) scores before and after forest therapy. T-A, tension-anxiety; C, confusion; A-H, anger-hostility; D, depression; F, fatigue; V, vigor; TMD, Total Mood Disturbance. N = 8, mean \pm standard error. ** p < 0.01, * p < 0.05, Wilcoxon signed-rank test.

4. Discussion

This study assessed the physiological and psychological benefits of forest therapy on middle-aged Japanese men with high-normal blood pressure. Japanese guidelines for the management of hypertension

(2014) [13] classify less than 140/90 mmHg as normal blood pressure and over 140/90 mmHg as high blood pressure. We enrolled only subjects diagnosed with "high-normal blood pressure" according to this definition. In general, the results were consistent with previous studies showing that forest therapy reduces multiple physiological and psychological indices of stress in healthy young adults [2,6,7]. Moreover, Li *et al.* reported that forest bathing significantly increased NK activity and decreased the concentration of adrenaline in urine, while a city tourist visit had no such effects [14]. However, the activities included in their forest therapy program were impossible to perform in an urban area, including meditation in front of a waterfall, embracing a tree, and the act of thinning of forest experiences. While the current study was preliminary in that we had no control group (*i.e.*, both visiting the forest and visiting an urban area were not compared), we provide evidence for both physiological and psychological benefits in middle-aged patients at risk for hypertension. Regular forest therapy may thus prevent progression to clinical hypertension, a possibility warranting further investigation.

As blood pressure and many other physiological indices show a circadian rhythm, we paid special care to conduct pre- and post-treatment measurements at the same time (mid-afternoon) on successive days. Thus, circadian variation did not contribute to the changes reported. Forest therapy (including a leisurely walk and relaxation in a forest) reduced systolic blood pressure, urinary adrenaline, and serum cortisol. Blood pressure is under dual regulation by the sympathetic and parasympathetic nervous systems, with sympathetic activity increasing and parasympathetic activity reducing blood pressure [15]. Sympathetic activity can be determined by measuring the levels of urinary adrenaline and/or noradrenaline [16], and there are significant correlations between blood pressure and both urinary adrenaline and noradrenaline [15]. Moreover, many previous studies have shown that reducing stress decreases systemic cortisol [17] and sympathetic activity [17]. Thus, forest therapy may lower systolic and diastolic blood pressure of middle-aged males with high normal blood pressures by reducing sympathetic activity, consistent with previous studies on young healthy adults using multiple measures of stress response and autonomic activity, including cortisol and heart rate variability [3,5,6].

According to the SD and POMS questionnaires, participants felt more "comfortable," "natural," and "relaxed" after forest therapy. In addition, negative emotions were significantly reduced. Similarly, younger healthy subjects reported being significantly more comfortable and calm after walking in a forest compared to urban walks [18].

The risks of all cardiovascular diseases, strokes, myocardial infarction, chronic kidney disease, and associated risks of mortality increase in parallel with blood pressure above the optimum [19]. Thus, even patients with high-normal blood pressure benefit from methods that lower blood pressure. This patient group does not need antihypertensive agents; however, modification of lifestyles factors (such as a high sodium diet), weight loss, and exercise are recommended. The current study suggests that regular forest therapy is a convenient option to lower blood pressure into the optimal range and possibly to prevent progression to hypertension and associated complications.

From the viewpoint of public health, it is necessary to shift blood pressure downward in the entire population and not only in high-risk hypertensive patients [20]. Because forests occupy 67% of the land in Japan, they are easily accessible. Thus, forest therapy can be an effective and beneficial treatment for people of all ages and backgrounds. It is expected that broader application of forest therapy will improve the general health of the nation and reduce public medical expenses.

The present study provides evidence of physiological and psychological benefits of forest therapy for middle-aged males with high-normal blood pressure. However, the limitations of the present study include a lack of a control group performing similar activities in an urban environment. Furthermore, these results cannot yet be extrapolated to females or hypertensive adults. Studies examining health benefits in these groups are warranted in future study.

5. Conclusions

Our study revealed that forest therapy elicited a significant: (1) decrease in systolic and diastolic blood pressure; (2) decrease in urinary adrenaline and serum cortisol levels; (3) increase in "relaxed" and "natural" feelings as assessed by the modified SD method; and (4) decrease in POMS negative subscales "tension-anxiety," "confusion," and "anger-hostility" as well as the TMD score in middle-aged males with high-normal blood pressure. Forest therapy may prevent progression to hypertension, thereby reducing associated risks of cardiovascular and renal diseases in this patient group.

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Author Contributions

Hiroko Ochiai contributed to data acquisition, interpretation of results, and manuscript preparation. Harumi Ikei and Chorong Song contributed to the experimental design, data acquisition, statistical analysis, and interpretation of results. Maiko Kobayashi and Ako Takamatsu conducted data acquisition. Takashi Miura contributed to preparing the experimental sites and cooperated with data acquisition. Takahide Kagawa and Qing Li participated in data acquisition and contributed to the interpretation of results. Shigeyoshi Kumeda and Michiko Imai conceived the study and participated in the interpretation of results. Yoshifumi Miyazaki conceived and designed the study, contributed to the interpretation of results, and manuscript preparation. All authors have read and approved the final version submitted for publication.

Conflicts of Interest

The authors declare no conflict of interest.

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Communication Physiological and Psychological Effects of a Forest Therapy Program on Middle-Aged Females

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Abstract: The natural environment is increasingly recognized as an effective counter to urban stress, and "Forest Therapy" has recently attracted attention as a relaxation and stress management activity with demonstrated clinical efficacy. The present study assessed the physiological and psychological effects of a forest therapy program on middle-aged females. Seventeen Japanese females (62.2 \pm 9.4 years; mean \pm standard deviation) participated in this experiment. Pulse rate, salivary cortisol level, and psychological indices were measured on the day before forest therapy and on the forest therapy day. Pulse rate and salivary cortisol were significantly lower than baseline following forest therapy, indicating that subjects were in a physiologically relaxed state. Subjects reported feeling significantly more "comfortable," "relaxed," and "natural" according to the semantic differential (SD) method. The Profile of Mood State (POMS) negative mood subscale score for "tension-anxiety" was significantly lower, while that for "vigor" was significantly higher following forest therapy. Our study revealed that forest therapy elicited a significant (1) decrease in pulse rate, (2) decrease in salivary cortisol levels, (3) increase in positive feelings, and (4) decrease in negative feelings. In conclusion, there are substantial physiological and psychological benefits of forest therapy on middle-aged females.

Keywords: forest therapy program; middle-aged females; pulse rate; salivary cortisol; semantic differential method; Profile of Mood State

1. Introduction

The term "forest bathing" was proposed in Japan in 1982, and penetrated as words to express for enjoying the comfort of the forest. However, there was little information regarding "What is so psychologically comforting about the forest?" and "What specific psychological and physiological changes are taking place in a body in the forest?" The elucidation of the phenomenon rapidly advanced around the past 10 years, and it developed into the term "forest therapy" programs. Indeed, "forest therapy" is now increasingly recognized as an effective relaxation and stress management

activity with demonstrated a preventive medical effect and increased healthy effect among healthy Japanese adults [1].

Several studies have shown that time spent in a forest can decrease blood pressure (BP) [2–6], pulse rate [2–7], sympathetic nervous activity [4–6,8–10], and cortisol levels [2–5,7,8,11,12], while increasing parasympathetic nervous activity [3–10]. Furthermore, forest stimulation decreased cerebral blood flow in the prefrontal cortex [12], and Bratman *et al.* reported that a brief nature experience decreased both self-reported rumination and neural activity in the subgenual prefrontal cortex (sgPFC) [13]. These studies suggest that accessible natural areas are a critical resource for improving mental health in our rapidly urbanizing world [13].

It was also shown that a forest therapy trip can increase human natural killer (NK) cell activity and improve immunity in both males and females, and these effects were found to last for at least 7 days [14–17]. Additionally, psychological studies have demonstrated that the negative mood was significantly lower and the positive mood was significantly higher after durations of stay in the forest [10,18].

Park *et al.* reported relaxation and stress-management effects of forest environments using several questionnaire-based metrics, in addition to improved mood [19]. In psychological tests of young adult males, forest therapy significantly increased positive feelings and reduced negative feelings in comparison with urban stimuli [2–4,6,8–12]. A majority of studies involving forest therapy experiments report the various effects in male subjects [4,8–10,19–21]; however, few reports have focused on female subjects [16].

Most field experiments on forest therapy have enrolled only healthy young adults as subjects, while those who need these benefits the most may be older adults at a higher risk of stress- and lifestyle-related diseases such as high BP, diabetes, and depression. Song *et al.* reported physiological and psychological relaxation effects on hypertensive individuals after a brief walk in the forest [20]; however, few studies have examined the effects of a standardized forest therapy program on higher-risk populations, particularly a program that can be completed within a day for convenience and broad accessibility. To address these issues, we planned experiments to measure the effects of a standardized forest therapy program on middle-aged males with high-normal BP [21] and found that systolic and diastolic BP, urinary adrenaline, and serum cortisol levels were significantly lower than baseline following the program. While this study lacked a control group, it did provide evidence that the physical and psychological benefits of a brief forest therapy program extend to middle-aged males. Here, we investigated the physiological and psychological effects of a standard forest therapy program on middle-aged females (mean age: 62 years) to allow comparison with the previously measured effects on male subjects of similar age.

2. Experimental Section

2.1. Subjects

Seventeen Japanese females ranging in age from 40 to 73 years (62.2 ± 9.4 years; mean \pm standard deviation) were recruited from the Health Promotion Center in Agematsu, Nagano Prefecture. Inclusion criteria were female aged 40 years or older. Candidates who thought it may be difficult to walk in hot weather were excluded. Six subjects were on medication for hypertension, which was well controlled. All participants were free from other diseases and psychological disorder. Body mass index (BMI) [22,23] was calculated from height and weight (BMI = weight (kg) \div {height (m) \times height (m)) and divided into a BMI \geq 25 group and a BMI < 25 group. At 14:00 on the day before the initiation of forest therapy, the subjects gathered in a waiting room at the Health Promotion Center; they were completely informed regarding the study aims and procedures before initiating the experiment. They received a description of the experiment, and all the subjects signed an agreement to participate. After physiological inspections and questionnaires were completed, the subjects disbanded at 16:30. To control for the effects of alcohol, the subjects did not consume alcohol during the entire study

period. Participants were directed to perform normal "everyday life" activities on the day before forest therapy. This study was approved by the Ethics Committee of Nagano Prefecture Kiso Hospital and the Center for Environment, Health and Field Sciences, Chiba University, Japan, on 19 August 2013 and performed according to the Declaration of Helsinki [24].

2.2. Experimental Sites

The forest therapy phase was conducted in Akasawa Shizen Kyuyourin (Akasawa Natural Recreation Forest), Agematsu, Nagano Prefecture (situated in central Japan) on 30 August 2014. The distance from the health promotion center to the forest was 14.6 km, and it took 42 min to drive by car. The weather was cloudy on the forest therapy day, with a mean temperature of 21.5 °C (18.2 °C–27.5 °C) and humidity of 81% (49%–96%).

2.3. Physiological Indices

Both systolic and diastolic BP levels and pulse rate readings were obtained from the right arm using a portable digital sphygmomanometer (HEM-1020, Omron, Kyoto, Japan). These procedures were performed between 15:09 and 15:22 on the day before forest therapy and between 14:44 and 14:56 after forest therapy to control for circadian effects.

Salivary cortisol, which shows a reliable increase under stress, was measured as an index of endocrine activity. Saliva samples were collected using a saliva collection aid (No.61/524,096; SalivaBio LLC, California, USA) between 15:28 and 15:35 on the day before forest therapy and between 14:57 and 15:05 after forest therapy. Saliva samples collected at the field site were immediately placed in a freezer and sent to a laboratory (MACROPHI Inc, Takamatsu, Japan) for analysis.

2.4. Psychological Indices

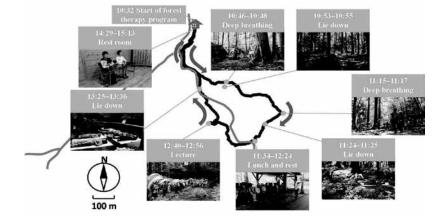
The semantic differential (SD) method and a short form of the Profile of Mood State (POMS) were used to evaluate psychological responses to forest therapy. These questionnaires were completed by subjects between 15:00 and 15:20 on the day before forest therapy and between 14:44 and 14:56 after forest therapy. The SD method uses three pairs of adjectives anchoring seven-point scales: "comfortable to uncomfortable," "relaxed to awakening," and "natural to artificial" [25]. The short form of POMS was used to decrease the burden on the subjects [26]. We assessed three subscales: "tension–anxiety," "fatigue," and "vigor."

2.5. Experimental Design

The subjects spent the previous night in their respective homes. On the morning of the forest therapy day, the subjects gathered in the same meeting room at 9:00 and participated in the forest therapy program as a group with a guide. They were not permitted to carry cell phones. The program consisted of multiple timed activities over 4 h and 41 min (Table 1) led by a guide. The subjects walked around their assigned area and then sat and lay on their backs in the forest on waterproof sheets laid on the ground during rest breaks. The guide put on measuring equipment with a map-caching offline GPS application (Geographica, Japan) and accompanied the subjects in the forest (Figure 1a,b).

Time	Event	Calorie Consumption (Kcal/min)
10:32-10:45	Stroll (Forest)	1.21
10:46-10:48	Deep breathing (Forest)	0
10:49-10:52	Stroll (Forest)	0.15
10:53-10:55	Lie down (Forest)	0
10:56-11:14	Stroll (Forest)	0.65
11:15-11:17	Deep breathing (Forest)	0.10
11:18-11:23	Stroll (Forest)	0.48
11:24-11:25	Lie down (Forest)	0.06
11:26-11:33	Stroll (Forest)	0.52
11:34-12:24	Lunch and rest (Resting room)	0.04
12:25-12:39	Stroll (Forest)	0.92
12:40-12:56	Lecture (Forest)	0.08
12:57-13:09	Stroll (Forest)	0.66
13:10-13:24	Rest (Forest)	0.01
13:25-13:36	Lie down & abdominal breathing (Forest)	0.00
13:37-13:59	Chat (Forest)	0.01
14:00-14:28	Stroll (Forest)	0.76
14:29–15:13	Rest (Resting room)	0.02

Table 1. Time schedules and calorie consumption during various activities of the forest therapy program.



(a)

1160 12:40-12:56 11:34-12:24 11:15-11:17 1140 10:46-10:48 10:53-10:55 11:24-11:25 13:25-13:36 14:29-15:13 1120 1100 Altitude(m) 1080 1060 1040 1020 1000 – 0 250 2,000 500 750 1,000 Distance(m) 1,250 1,500 1,750 **(b**)

Figure 1. Images showing the various activities of the forest therapy program with location map. (a) : plane map, (b): altitude map.

Energy expenditure was assessed for each activity using Lifecorder GS4 (Suzuken Co., Ltd., Chiba, Japan). Tobacco and all drinks (except mineral water) were prohibited during forest therapy. The subjects ate the same lunch made from local ingredients at the same time (11:34–12:24). After the subjects completed the program, they returned to a waiting room for post-treatment measurements and to complete the questionnaires. These results were then compared with those obtained on the previous day.

We aimed to compare the physiological and psychological effects of forest therapy with everyday life activities on a normal day. Physiological and psychological inspections were performed at approximately the same time on the day before and on the day of the therapy.

2.6. Statistical Analysis

We used paired *t*-tests to compare physiological indices and the Wilcoxon signed-rank test to compare psychological test results obtained before and immediately after forest therapy. All statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). Data are expressed as the mean \pm standard error (mean \pm SE). For all tests, *p* < 0.05 (one sided) was considered statistically significant.

3. Results

Pulse rate was significantly lower after forest therapy than on the day before forest therapy (baseline) in middle-aged females (69.1 \pm 2.7 *vs*. 73.1 \pm 2.5 beats/min; t(16) = 4.67, *p* < 0.01 by paired *t*-test) (Figure 2). Similarly, salivary cortisol levels were significantly lower after forest therapy than on the day before forest therapy (0.124 \pm 0.009 *vs*. 0.168 \pm 0. 020 µg/dL; t(16) = 2.63, *p* < 0.05 by paired *t*-test) (Figure 3).

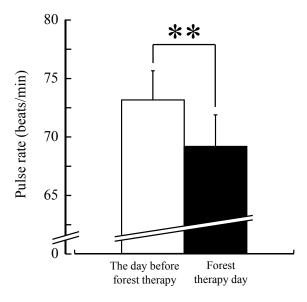


Figure 2. Effect of forest therapy on pulse rate of middle-aged females. N = 17, mean \pm standard error. ** *p* < 0.01, paired *t*-test.

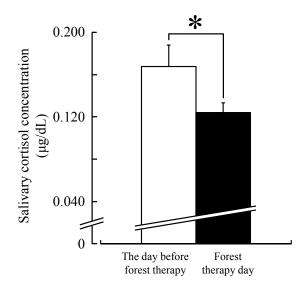


Figure 3. Effect of forest therapy on salivary cortisol level. N = 17, mean \pm standard error. * *p* < 0.05, paired *t*-test.

The total energy expenditure during forest therapy was compared between subjects with BMI ≥ 25 (N = 4) and those with BMI < 25 (N = 13). A marginally significant difference was observed between groups, with 24% greater expenditure in the BMI ≥ 25 group compared with the BMI < 25 group (0.88 \pm 0.08 *vs*. 0.71 \pm 0.06 kcal/min; t(15) = 1.88, *p* < 0.10 by unpaired *t*-test). The mean salivary cortisol level was reduced in the BMI < 25 group after forest therapy (0.186 \pm 0.024 *vs*. 0.123 \pm 0.012µg/dL; t(12) = 3.19, *p* < 0.01 by paired *t*-test), but it actually increased slightly in the BMI ≥ 25 group (0.109 \pm 0.013 *vs*. 0.128 \pm 0.014µg/dL; t(3) = 4.01, *p* < 0.05 by paired *t*-test).

Significantly higher SD scores were observed for the adjectives "comfortable" (p < 0.01), "relaxed" (p < 0.01), and "natural" (p < 0.01) after forest therapy than on the day before forest therapy (Figure 4). Finally, a significant elevation of mood was detected on POMS (Figure 5), with scores for the negative subscale "tension–anxiety" being significantly lower (p < 0.01) and those for the positive subscale "vigor" (p < 0.01) being significantly higher after forest therapy.

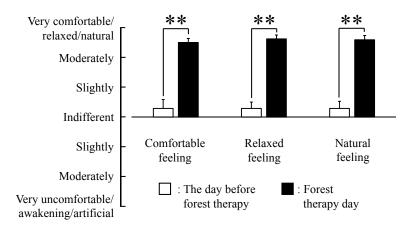


Figure 4. Semantic differential (SD) method scores for the day before forest therapy and immediately after forest therapy, showing changes in the subjective feelings "comfortable," "relaxed," and "natural". N = 17, mean \pm standard error. ** *p* < 0.01, Wilcoxon signed-rank test.

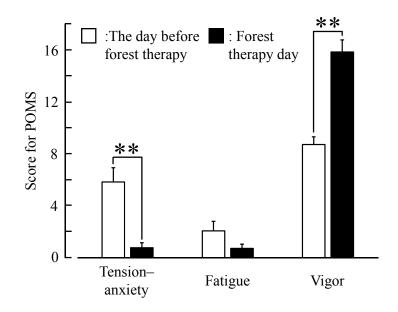


Figure 5. Lower negative and higher positive subjective Profile of Mood State (POMS) subscores after forest therapy than on the day before forest therapy. N = 17, mean \pm standard error. ** *p* < 0.01, Wilcoxon signed-rank test.

4. Discussion

The present study assessed the physiological and psychological benefits of forest therapy on middle-aged Japanese females. The mean pulse rate was significantly lower after walking in a forest environment than on the day before forest therapy. Because the pulse rate is a basic index of autonomic nervous system activation, the drop in pulse rate indicates a state of relaxation in middle-aged females, consistent with the past reports that examined physiological responses to a natural environment in young adults [2–7]. Thus, we concluded that this benefit of physiological relaxation extends to middle-aged females.

Sympathetic activity can be determined by measuring the levels of urinary adrenaline and/or noradrenaline [27], and many previous studies have shown that reducing stress decreases sympathetic activity, as measured by systemic cortisol levels [28]. The concentration of cortisol was the highest immediately after waking up and decreases and stabilizes in the afternoon. We used saliva samples for measuring cortisol levels as this method is easily manageable in a field setting and is non-invasive. Furthermore, salivary cortisol provides a reliable prediction of total and calculated free serum cortisol levels [29]. It has been reported that the normal level of salivary cortisol is $0.07-0.73 \mu g/dL$ [30]. Many previous studies have shown that lowered stress levels result in lower cortisol levels [2–5,7,8,11,12]; therefore, we conclude that forest therapy also reduces stress in middle-aged females.

For several decades, BMI (kg/m²) has been used to diagnose obesity in clinical practice and obesity research and to structure programs and goals for weight loss interventions [31]. BMI is sometimes used to estimate total body fat and determine whether a person has a healthy weight. While BMI may not always provide an accurate estimate of excess body fat, BMI \ge 25 is linked to increased risk of diseases such as heart disease and some cancers. In the present study, the salivary cortisol level was reduced only in subjects with BMI < 25. Note, however, that these measures are derived for a single forest therapy session, which may have been more stressful on the heavier subjects. Therefore, for middle-aged females with high BMI, a sustained regular program may be necessary for the anti-stress benefits to emerge.

The baseline salivary cortisol level was 1.7-fold higher in subjects with BMI < 25 than in those with BMI \ge 25, but this difference disappeared after forest therapy. Song *et al.* reported that subjects

with high initial BP showed a decrease, while those with low initial values showed an increase after walking in a forest area [32]. These results suggest a physiological adjustment effect in the forest environment, which may also account for the normalization of cortisol levels among participants with different BMI. However, no report has studied this adjustment effect for cortisol levels in subjects matched for baseline BMI; therefore, additional studies are necessary.

According to the SD questionnaires, middle-aged females felt more "comfortable," "natural," and "relaxed" after forest therapy. In addition, the negative emotion "tension–anxiety" was reduced and the positive feeling of "vigor" was higher after forest therapy according to the short form of POMS. Similarly, middle-aged males reported feeling significantly more "natural" and "relaxed" after walking in a forest [21]. While "tension–anxiety" was significantly lower after forest therapy in middle-aged males as well, in contrast to middle-aged females, they reported no significant change in "vigor" [21]. Neither group reported changes in "fatigue," although measurement immediately after the forest walk may have contributed to temporary fatigue. Nonetheless, these findings indicate that a single forest therapy session has psychological benefits for both middle-aged women and men.

Although many factors can affect the general condition of menstruating females, little is known about differences in the relationship between physiological and subjective stress responses at various phases of the menstrual cycle [33]. Watanabe *et al.* reported that no significant differences in salivary cortisol levels were observed during any phase of the menstrual cycle [34]. Additionally, the mean age of this study sample was 62 years. However, because the mean age for menopause in Japanese women is approximately 50 years, we did not consider the influence of the menstrual cycle in this experiment. Menopausal disorders are a frequent problem in middle-aged females. Many of these problems may stem from disruption of the intricate links between estrogen metabolism and the autonomic nervous system. Many women gain weight because of the decrease in estrogen and basal metabolism, while autonomic changes may lead to tachycardia and mental health effects. Normal aging influences various indices, and the parasympathetic tone is generally higher in women than men, as evidenced by heart rate variability (HRV) measurements [35]. It has been reported that physical activity facilitates improved HRV stability in older women and that the quantity of exercise training necessary for such an improvement is relatively modest [36].

"Forest therapy" is increasingly recognized not only as a convenient exercise but also as a relaxation and stress management activity with demonstrated clinical benefits [1]. Moreover, we can control the energy expenditure by choosing the appropriate course terrain, distance, and walking speed and by including regular rest and relaxation sessions, such as sitting, lying, and deep breathing. Forest therapy could be an effective and convenient method for the improvement of menopausal symptoms such as autonomic imbalance, stiff shoulder, knee pain, constipation, shortness of breath, and depression. Furthermore, as a group activity, forest therapy is an opportunity to spend time enjoying the natural environment with friends and family.

The present study provides evidence for physiological and psychological benefits of forest therapy in middle-aged females. Limitations of the present study include the lack of a control group performing similar activities in an urban environment. An ideal experimental design would include a comparison of the effects of forest therapy using the same parameters/environmental stimuli, but instead conducting the comparison (control group) in an urban area setting. However, this would be difficult to implement in practice because it involves activities such as "lying down" in an urban area. So the control experiments of the same activities completed indoors are thought to be necessary in future. Interestingly, differences in effect were observed with varying BMI. However, the limited number of subjects in this study decreased the significance of the analysis. Future studies should include a larger number of subjects. Furthermore, forest therapy has not yet been shown to actually reduce the risk of disease independent of the general effects of exercise. It is now necessary to design experiments that test whether forest therapy can reduce disease risk in vulnerable populations through these demonstrated physiological and psychological benefits.

5. Conclusions

Our study revealed that forest therapy elicited a significant (1) decrease in pulse rate, (2) decrease in salivary cortisol levels, (3) increase in "comfortable," "natural," and "relaxed" feelings as assessed by the modified SD method, (4) decrease in the POMS negative subscale "tension–anxiety," and (5) increase in feelings of "vigor" in middle-aged females. In conclusion, walking in a forest according to a standard "forest therapy" program induced physiological and psychological relaxation. These results clarified the physiological effects of the forest therapy program and suggested a possibility of clinical use.

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Author Contributions: Hiroko Ochiai contributed to data acquisition, interpretation of results, and manuscript preparation. Harumi Ikei and Chorong Song contributed to the experimental design, data acquisition, statistical analysis, and interpretation of results. Maiko Kobayashi conducted data acquisition. Takashi Miura contributed to preparation of the experimental sites and cooperated with data acquisition. Takahide Kagawa and Qing Li participated in data acquisition and contributed to the interpretation of results. Shigeyoshi Kumeda and Michiko Imai conceived the study and participated in the interpretation of results. Yoshifumi Miyazaki conceived and designed the study and contributed to the interpretation of results and manuscript preparation. All authors have read and approved the final version submitted for publication.

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Research Article

Effects of Forest Bathing on Cardiovascular and Metabolic Parameters in Middle-Aged Males

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In the present study, we investigated the effects of a forest bathing on cardiovascular and metabolic parameters. Nineteen middleaged male subjects were selected after they provided informed consent. These subjects took day trips to a forest park in Agematsu, Nagano Prefecture, and to an urban area of Nagano Prefecture as control in August 2015. On both trips, they walked 2.6 km for 80 min each in the morning and afternoon on Saturdays. Blood and urine were sampled before and after each trip. Cardiovascular and metabolic parameters were measured. Blood pressure and pulse rate were measured during the trips. The Japanese version of the profile of mood states (POMS) test was conducted before, during, and after the trips. Ambient temperature and humidity were monitored during the trips. The forest bathing program significantly reduced pulse rate and significantly increased the score for vigor and decreased the scores for depression, fatigue, anxiety, and confusion. Urinary adrenaline after forest bathing showed a tendency toward decrease. Urinary dopamine after forest bathing was significantly lower than that after urban area walking, suggesting the relaxing effect of the forest bathing. Serum adiponectin after the forest bathing was significantly greater than that after urban area walking.

1. Introduction

The forest environment has long been enjoyed for its quiet atmosphere, beautiful scenery, calm climate, pleasant aromas, and clean fresh air. Researchers in Japan have tried to find preventive effects against lifestyle-related diseases from forests and have proposed a new concept called "forest bathing." What is forest bathing? In Japan, a forest bathing is a short leisurely visit to a forest, called "*Shinrin-yoku*" in Japanese, which is similar in effect to natural aromatherapy, for the purpose of relaxation. "*Shinrin*" means forest and "*yoku*" means bathing in Japanese [1, 2]. Since forests occupy 67% of the land in Japan, forest bathing is easily accessible. Forest bathing as a recognized relaxation and/or stress management activity and a method of preventing diseases and promoting health is becoming a focus of public attention in Japan [2].

We previously found that forest bathing enhances human natural killer (NK) activity by increasing the number of NK cells and intracellular levels of anticancer proteins such as perforin, granulysin, and granzymes in both male and female subjects [1–5]. The increased NK activity was shown to last for more than 30 days after a trip [3, 4]. This has very important implications for preventive medicine. Conversely, taking an urban trip has not been shown to increase human NK

TABLE 1: Information of the subjects.

Number	Age (year)	Height (cm)	Body weight (kg)	BMI	SBP (mmHg)	DBP (mmHg)	Pulse rate/min	Remark [#]	Smoking status
1	69	168	57	20	142	83	63	Stage I HT	No
2	67	179	72	22	138	85	60	High-normal	No
3	40	184	87	26	127	81	92	Normal	Smoking
4	56	176	62	20	148	84	101	Stage I HT	No
5	44	166	74	29	144	93	73	Stage I HT	No
6	46	180	68	21	143	92	79	Stage I HT	Smoking
7	55	167	54	19	157	103	81	Stage II HT	No
8	49	173	65	22	150	99	62	Stage I HT	No
9	40	179	84	26	146	99	68	Stage I HT	No
10	44	172	74	25	148	92	80	Stage I HT	Smoking
11	46	184	70	21	143	84	96	Stage I HT	Smoking
12	46	165	58	21	147	98	70	Stage I HT	No
13	49	172	78	26	149	104	79	Stage I HT	No
14	66	172	70	23	161	95	61	Stage II HT	No
15	50	170	63	22	155	106	64	Stage II HT	Smoking
16	44	170	102	35	131	93	75	Stage I HT	No
17	45	165	61	22	141	84	60	Stage I HT	No
18	59	176	70	23	140	90	64	Stage I HT	No
19	58	182	85	26	126	94	67	Stage I HT	No
Mean	51.2	173.7	71.2	23.7	144.0	92.6	73.4		
SD	8.8	6.1	11.7	3.7	9.0	7.4	12.1		
SE	2.0	1.4	2.7	0.9	2.1	1.7	2.8		

[#]Based on the Japanese Society of Hypertension Guidelines [11]. HT: hypertension.

activity, numbers of NK cells, or the expression of the selected intracellular perforin, granulysin, and granzymes A/B, indicating that increased NK activity during forest bathing is not due to the trip itself but due to the forest environment [3]. Moreover, forest bathing reduces sympathetic nervous activity and negative emotions, increases parasympathetic nervous activity, and has a relaxing effect on humans [1, 2, 4– 9]. Although there have been several studies with healthy young adults as subjects [6–8], few studies have investigated the effects of forest bathing on middle-aged subjects [9]. It is generally accepted that studying the effect of walking in a forest environment on cardiovascular function in middleaged subjects is more important than that in young male students, and even more so for subjects with higher blood pressure.

Based on the findings mentioned above, because forest environments reduce sympathetic nervous activity and increase parasympathetic nervous activity, we speculated that walking in a forest environment may have beneficial effects on cardiovascular function. Thus, in the present study, we investigated the effects of walking in a forest park on cardiovascular and metabolic parameters in middle-aged males.

2. Subjects and Methods

2.1. Subjects. In the present study, we investigated the effects of forest bathing on blood pressure and pulse rate during walking by an ambulatory automatic blood pressure

monitor and other cardiovascular and metabolic parameters in middle-aged male subjects. Nineteen middle-aged male subjects, ranging in age from 40 to 69 years (mean \pm SD: 51.2 ± 8.8), were recruited for the present study (Table 1). Advertisements were placed in newspapers to recruit the subjects with the following conditions: (1) males whose ages are between 40 and 74 years old, (2) people with highnormal or hypertension, and (3) people not taking any antihypertensive drugs. The subjects live and work in small cities. Although the levels of systolic and diastolic blood pressure were 144.0±9.9 mmHg and 92.6±7.4 mmHg, respectively, these subjects were not taking any antihypertensive drugs. Information about the subjects was gathered from a self-administered questionnaire that asked about cigarette smoking, alcohol consumption, and sleeping hours and has been reported previously [9, 10]. Written informed consent was obtained from all subjects after a full explanation of the study procedures. None of the subjects had any symptoms of disease, used drugs that might have affected the results, or were taking any medication at the time of the study. The subjects took the same breakfast and lunch during the two trips. To control for the effects of alcohol, the subjects did not consume alcohol during the study period. The Ethics Committees of the Nippon Medical School and Nagano Prefectural Kiso Hospital approved this study.

2.2. Walking in a Forest Environment and in an Urban Area. We previously found that the effects of walking in forest environments on the immune function (natural killer activity) Evidence-Based Complementary and Alternative Medicine



FIGURE 1: Forest bathing.



FIGURE 2: Urban area walking.

lasted for more than one week, and sometimes even 4 weeks, but not walking in urban environments [3–5]. Therefore, to avoid such lasting effects, we designed the study so that all subjects first walked in the urban area and then in the forest. The interval between the two experiments was one week. The subjects took day trips to a forest park named Akasawa Shizen Kyuyourin (Akasawa Natural Recreation Forest) in Agematsu, Nagano Prefecture (situated in central Japan) (Figure 1) on August 29, 2015, and to an urban area of Nagano Prefecture where there were almost no trees as a control (Figure 2) on August 22, 2015. On both trips, they walked 2.6 km for 80 min with the same speed guided by the some guide in the morning (11:00-12:20) and afternoon (13:40-15:00) on Saturdays. The subjects did not communicate with each other during the walk to avoid the effects of talking. Both waking courses are the flat walking ways without any slope. To control for the effects of cigarette smoking, the smokers did not smoke during the walking. To control for the effects of caffeine, the subjects were only allowed to drink mineral water during the walking.

2.3. Physiological and Psychological Indices. Blood and urine were sampled in the morning before and after each trip. Cardiovascular and metabolic parameters were measured. Blood pressure and pulse rate were measured by an ambulatory automatic blood pressure monitor at the same time every 20 min during each trip. The Japanese version of the profile of mood states (POMS) test was conducted before, during, and after the trips [1]. Ambient temperature and humidity were monitored during the trips.

2.4. Blood Analysis [9]. The serum levels of triglycerides, total cholesterol (Cho), low density lipoprotein (LDL) Cho, high density lipoprotein (HDL) Cho, and remnant-like particles (RLP) Cho were analyzed using an enzymatic assay with an autoanalyzer. The serum total adiponectin concentration was measured using an enzyme immunoassay (EIA). Blood glucose concentration was analyzed using a Glucocard GT-1640 and Diasenser strips (Arkray, Kyoto, Japan). The serum level of insulin was analyzed using a chemiluminescent immunometric assay (CLIA), and the serum level of dehydroepiandrosterone sulfate (DHEA-S) was analyzed using a chemiluminescence enzyme immunoassay (CLEIA). The serum level of high-sensitivity C-reactive protein (hs-CRP) was analyzed using a latex nephelometric assay.

2.5. Urinary Adrenaline, Noradrenaline, and Dopamine Measurements. The levels of adrenaline, noradrenaline, and dopamine in urine were measured by an HPLC method using an HLC-725CAII analyzer. The instrument features a column-switching system composed of two pretreatment columns, a separation column, and a high-sensitivity detection unit based on a postcolumn reaction using the fluorogenic reagent 1,2-diphenylethylenediamine. The detection limits of adrenaline, noradrenaline, and dopamine in urine were all 8 fmol/mL [3–5]. The values of urinary adrenaline, noradrenaline, and dopamine were corrected by urinary creatinine and indicated as $\mu g/g$ creatinine.

2.6. Statistical Analysis. The paired *t*-test was used to compare the differences between urban and forest environments. The differences between before and after the walking in some results were also compared by the paired *t*-test. The analyses were performed with the Microsoft Excel software package for Windows. The significance level for p values was set at 0.05.

3. Results

It was sunny weather in the urban trip, whereas it was rainy weather in the morning and was cloudy in the afternoon in the forest bathing. The respective maximum and average temperatures and average humidity were 32.7° C, $31.2\pm0.7^{\circ}$ C, and $52.4\pm2.6\%$ during the morning and 37.5° C, $33.2\pm1.4^{\circ}$ C, and $47.5\pm4.3\%$ during the afternoon in the urban area environment, whereas in the forest environment, the maximum and average temperatures and average humidity were 20.4° C, $19.1\pm0.5^{\circ}$ C, and $94.3\pm3.9\%$ during the afternoon, respectively.

As show in Table 1, the cohort demographics of 19 middleaged male subjects are as follows: (1) the average of age was 51.2 ± 8.8 years (mean \pm SD), (2) the levels of average systolic and diastolic blood pressure were 144.0 \pm 9.9 mmHg and 92.6 \pm 7.4 mmHg, respectively, (3) the average of pulse rate was 73.4 \pm 12.1 bpm, (4) the average of height was 173.7 \pm 6.1 cm,

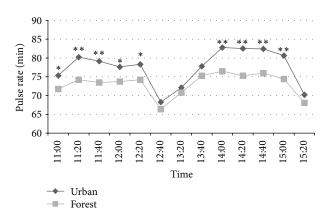


FIGURE 3: Forest bathing program significantly reduces the pulse rate in male subjects. *p < 0.05, **p < 0.01, forest versus urban by paired *t*-test (n = 19).

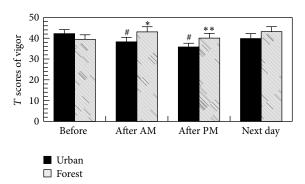


FIGURE 4: Effects of forest bathing on the *T* scores of vigor in the POMS test. *p < 0.05, **p < 0.01, versus urban, and *p < 0.01 versus before by paired *t*-test (mean + SE, n = 19); after AM: after walking on the morning and after PM: after walking on the afternoon.

(5) the average of body weight was 71.2 ± 11.7 kg, (6) the average of BMI was 23.7 ± 3.7 , and (7) five subjects were smokers and 14 subjects were nonsmokers. These subjects were not taking any antihypertensive drugs.

3.1. Effects of Forest Bathing on Pulse Rate. As shown in Figure 3, forest bathing significantly reduced the subjects' pulse rate during 11:00–12:20 and 14:00–15:00, suggesting the relaxing effect of this program.

3.2. Effects of Forest Bathing on Feelings in POMS Test. As shown in Figure 4, the forest bathing significantly increased the score for vigor, whereas urban area walking significantly decreased the score for vigor in the POMS test, suggesting the relaxing effect of forest bathing program.

As shown in Figure 5, there was a significant decrease in the scores for anxiety in the POMS test after walking in the forest on the afternoon compared to that walking in the urban, suggesting the relaxing effect of forest bathing program.

As shown in Figure 6, the forest bathing significantly decreased the scores for fatigue, whereas urban area walking

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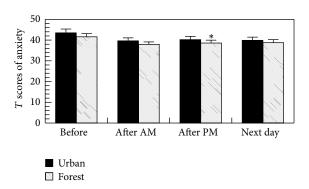


FIGURE 5: Effects of forest bathing on the *T* scores of anxiety in the POMS test. *p < 0.05 versus urban, by paired *t*-test (mean + SE, n = 19); after AM: after walking on the morning and after PM: after walking on the afternoon.

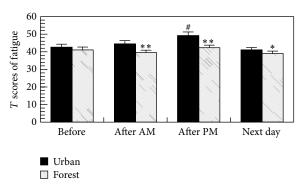


FIGURE 6: Effects of forest bathing on the *T* scores of fatigue in the POMS test. **p* < 0.05, ***p* < 0.01, versus urban, **p* < 0.01 versus before by paired *t*-test (mean + SE, *n* = 19); after AM: after walking on the morning and after PM: after walking on the afternoon.

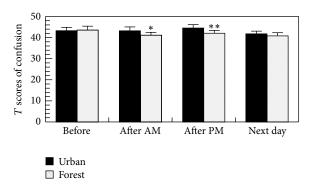


FIGURE 7: Effects of forest bathing on the *T* scores of confusion in the POMS test. * p < 0.05, ** p < 0.01, versus urban by paired *t*-test (mean + SE, n = 19); after AM: after walking on the morning and after PM: after walking on the afternoon.

significantly increased the score for fatigue in the POMS test, suggesting the relaxing effect of forest bathing program.

As shown in Figure 7, the forest bathing significantly decreased the scores for confusion in the POMS test, suggesting the relaxing effect of forest bathing program.

There was a significant decrease in the scores for depression in the POMS test after walking in the forest on the Evidence-Based Complementary and Alternative Medicine

TABLE 2: Effect of forest bathing on the levels of urinary noradrenaline, dopamine, and adrenaline in male subjects.

Before walking	After walking	
93.17 ± 6.57	$78.46 \pm 5.56^{\#}$	
$79.35 \pm 5.95^{*}$	$70.29 \pm 6.36^{*,\#}$	
503.65 ± 31.23	560.40 ± 39.25	
428.66 ± 28.59	$447.97 \pm 20.76^{**}$	
5.50 ± 0.84	5.371 ± 1.09	
5.04 ± 0.73	4.42 ± 0.63	
	93.17 ± 6.57 $79.35 \pm 5.95^{*}$ 503.65 ± 31.23 428.66 ± 28.59 5.50 ± 0.84	

Mean \pm SE, n = 19, *p < 0.05, **p < 0.01 versus urban; "p < 0.05, ##p < 0.01 versus before, by paired *t*-test.

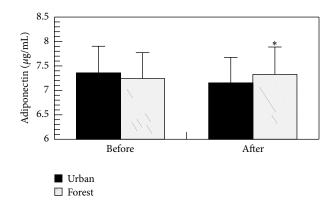


FIGURE 8: Effect of forest bathing program on the level of adiponectin in male subjects. *P < 0.05, versus urban by paired *t*-test (mean + SE, n = 19).

morning compared to that before the walking, suggesting the relaxing effect of forest bathing program (data not shown). Both trips did not affect the scores for angry (data not shown).

3.3. Effects of Forest Bathing on Urinary Adrenaline, Noradrenaline, and Dopamine. As shown in Table 2, both trips significantly reduced the level of urinary noradrenaline. Although there was no significant difference between before and after forest walking, the urinary adrenaline level showed a tendency toward decrease after forest walking. The urinary dopamine level after forest bathing was significantly lower than that after urban area walking; however, there was no difference in baseline (before the trips), suggesting that forest bathing may have a beneficial effect on urinary dopamine.

3.4. Effects of Forest Bathing on the Level of Adiponectin in Serum. As shown in Figure 8, the level of adiponectin in serum after forest bathing was significantly greater than that after urban area walking; however, there was no difference in baseline (before the trips), suggesting that forest bathing may have a beneficial effect on adiponectin in serum.

3.5. Effects of Forest Bathing on Blood Pressure. As shown in Figure 9, there was no significant difference in blood

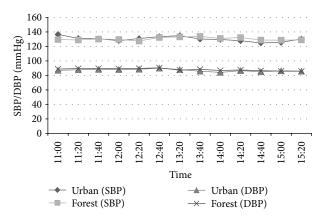


FIGURE 9: Effect of forest bathing on blood pressure level in male subjects (n = 19).

pressure between forest and urban area walking during the trips because of the big difference in ambient temperature between the forest (lower temperature) and urban area (higher temperature) environments.

3.6. Effects of Forest Bathing on Metabolic Parameters. Neither walking in the forest nor walking in the urban area affected the levels of triglycerides, total cholesterol (Cho), low density lipoprotein Cho, high density lipoprotein Cho, insulin, HbA1c, or high-sensitivity C-reactive protein in serum, or blood glucose. Both trips also had no effect on the numbers of white blood cells, red blood cells, and platelets, lymphocytes, granulocytes, or monocytes or the Hb concentration in the peripheral blood (data not shown).

4. Discussion

It is generally accepted that studying the effect of walking in forest environments on cardiovascular function in middleaged subjects is more important than that in young male students, especially in subjects with a higher blood pressure. However, few studies have investigated the effects of forest bathing on middle-aged hypertensive subjects [9]. Thus, in the present study we evaluated the effects of forest bathing on cardiovascular function in middle-aged hypertensive subjects.

We found that walking in a forest park significantly reduced the pulse rate in middle-aged males with higher blood pressure, compared with walking in an urban area. Because pulse rate is a basic index of activity of the autonomic nervous system, this decrease in pulse rate indicates a state of relaxation in the subjects. We previously also found that a forest bathing program significantly reduced the pulse rate in both middle-aged males [12] and females [13].

The forest bathing significantly increased the score for vigor and decreased the scores for depression, anxiety, fatigue, and confusion in the POMS test, whereas urban area walking significantly increased the score for fatigue and decreased the score for vigor, suggesting a relaxing effect of the forest bathing program. We previously also found that a forest bathing program significantly reduced the scores for depression, fatigue, anxiety, angry, and confusion and increased the score for vigor in the POMS test in both males [1, 2, 5–9] and females [2, 4, 13].

Why does forest bathing reduce the pulse rate and the scores for depression, fatigue, and confusion in the POMS test? To answer this question, we measured the levels of urinary adrenaline, noradrenaline, and dopamine in the present study. Although there was no significant difference between before and after forest walking, the urinary adrenaline level showed a tendency toward decrease after forest walking. The level of urinary dopamine after forest bathing was significantly lower than those after urban walking. It has been reported that sympathetic nerve activity can be determined by measuring the levels of urinary adrenaline, noradrenaline, and/or dopamine [14], suggesting that sympathetic nerve activity was lower during forest bathing. We previously found that forest bathing significantly reduced the levels of urinary adrenaline and noradrenaline in both male and female subjects [3-5, 9].

The level of adiponectin in serum after forest bathing was significantly greater than that after urban area walking; however, there was no difference in baseline (before the trips), suggesting that forest bathing may have a beneficial effect on adiponectin in serum. Adiponectin is a serum protein hormone that is specifically produced by adipose tissue. Studies have shown that lower-than-normal blood adiponectin concentrations are associated with several metabolic disorders, including obesity, type 2 diabetes mellitus, cardiovascular disease, and metabolic syndrome [15]. This result supports our previous finding that forest bathing significantly increased the serum adiponectin level in middle-aged males [9].

There was no significant difference in blood pressure between the forest and urban area walking during the trips because of the big difference in ambient temperature between the forest (lower temperature) and urban (higher temperature) environments. It has been reported that a higher ambient temperature reduces blood pressure, whereas a lower ambient temperature raises blood pressure [16-19]. Moreover, Woodhouse et al. [16] reported that the blood pressure of elderly people may be inversely related to the ambient temperature and that after adjustment for confounding seasonal effects, a 1°C decrease in living-room temperature was associated with rises of 1.3 mmHg in SBP and 0.6 mmHg in DBP. Hozawa et al. [18] also reported that when the outside temperature was ≥10°C, a 1°C increment of outside temperature corresponds to 0.4 mmHg and 0.28 mmHg decrease of SBP and DBP. Based on these findings, the blood pressure levels of subjects should have been higher in forest than in urban area because of the lower temperature in the forest; however, the blood pressure levels of subjects in the forest were almost the same as those in the urban area, suggesting that forest environment prevented the increase in blood pressure that should have been seen due to the lower temperature. In other words, the forest bathing contributed to the control of blood pressure and had a beneficial effect on blood pressure. Further studies should be conducted with the same ambient temperatures in forest and urban areas. In fact, we previously found that forest bathing significantly reduced

blood pressure by reducing sympathetic nerve activity and urinary adrenaline, and noradrenaline and dopamine levels; in that study the ambient temperature in the forest was almost the same as that in the urban area [9].

Neither walking in the forest nor walking in the urban area affected the levels of triglycerides, total cholesterol (Cho), low density lipoprotein Cho, high density lipoprotein Cho, insulin, HbA1c, or high-sensitivity C-reactive protein in serum, or blood glucose. Both trips also had no effect on the numbers of white blood cells, red blood cells, and platelets, lymphocytes, granulocytes, or monocytes or the Hb concentration in the peripheral blood (data not shown), which are similar to the previous study [9].

We previously found that the effects of walking in forest environments on the immune function (natural killer activity) lasted for more than one week, but not walking in urban environments [3–5]. Therefore, to avoid such lasting effects, we designed the study so that all subjects first walked in the urban area and then in the forest in the present study. Because there were no carry-over effects when subjects walked in an urban environment [3] the interval between the two experiments was one week.

The subjects did not communicate with each other during the walk to avoid the effects of talking. Therefore, although all participants completed the walks together it would not affect the results. However, the order of exposure (forest versus urban) is not counterbalanced and all participants completed the urban walk followed by the forest walk to avoid any carryover effects after forest bathing. This is a limitation of the present study.

It was very difficult to recruit the subjects in this study. We placed advertisements in newspapers to recruit the subjects. Maybe they are not representative of the broader population and an ideal recruitment of subjects would use a randomized stratification. This is another limitation of the present study.

5. Conclusions

Despite the bad weather conditions (rainy weather and lower ambient temperature) during the forest bathing, our study indicated that forest bathing produced the following significant benefits compared to urban area walking:

- (1) Decrease in pulse rate.
- (2) Decrease in urinary dopamine.
- (3) Tendency toward decrease in urinary adrenaline.
- (4) Increase in adiponectin in serum.
- (5) Decreases in negative moods such as anxiety, depression, fatigue, and confusion.
- (6) Increase in feelings of vigor in the POMS test in middle-aged males with higher blood pressure.

Taken together, the forest bathing program induced significant physiological and psychological relaxation. These findings clarified the physiological and psychological effects of the forest bathing program and suggested a possibility of clinical use. We recommend conducting forest bathing in a warmer day. Evidence-Based Complementary and Alternative Medicine

Competing Interests

The authors declare no conflict of interests.

Authors' Contributions

Qing Li conceived and designed the study and contributed to data acquisition, interpretation of results, and paper preparation. Maiko Kobayashi, Toshiya Ochiai, and Zhiyu Wang conducted data acquisition. Takashi Miura contributed to preparation of the experimental sites and cooperated with data acquisition. Takahide Kagawa participated in data acquisition and contributed to the interpretation of results. Shigeyoshi Kumeda, Michiko Imai, Toshiaki Otsuka, and Tomoyuki Kawada conceived the study and participated in the interpretation of results. All authors read and approved the final version submitted for publication.

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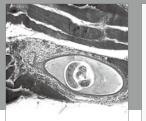
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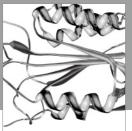


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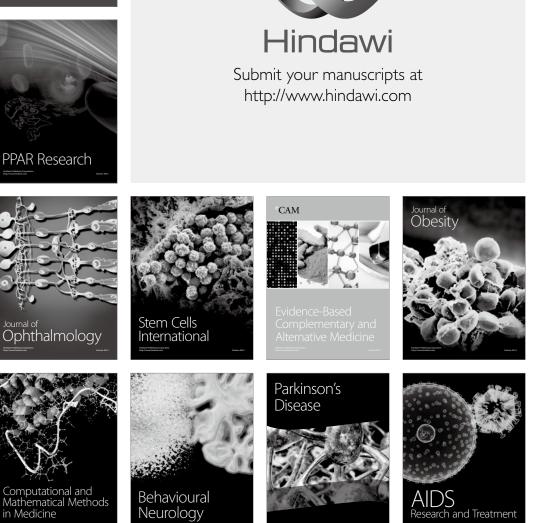


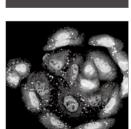
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Article Physiological Effects of Visual Stimulation with Forest Imagery

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Abstract: This study was aimed to clarify the physiological effects of visual stimulation using forest imagery on activity of the brain and autonomic nervous system. Seventeen female university students (mean age, 21.1 ± 1.0 years) participated in the study. As an indicator of brain activity, oxyhemoglobin (oxy-Hb) concentrations were measured in the left and right prefrontal cortex using near-infrared time-resolved spectroscopy. Heart rate variability (HRV) was used as an indicator of autonomic nervous activity. The high-frequency (HF) component of HRV, which reflected parasympathetic nervous activity, and the ratio of low-frequency (LF) and high-frequency components (LF/HF), which reflected sympathetic nervous activity, were measured. Forest and city (control) images were used as visual stimuli using a large plasma display window. After sitting at rest viewing a gray background for 60 s, participants viewed two images for 90 s. During rest and visual stimulation, HRV and oxy-Hb concentration in the prefrontal cortex were continuously measured. Immediately thereafter, subjective evaluation of feelings was performed using a modified semantic differential (SD) method. The results showed that visual stimulation with forest imagery induced (1) a significant decrease in oxy-Hb concentrations in the right prefrontal cortex and (2) a significant increase in perceptions of feeling "comfortable," "relaxed," and "natural."

Keywords: forest therapy; shinrin-yoku; forest imagery; autonomic nervous activity; prefrontal cortex activity; heart rate variability; near-infrared spectroscopy; semantic differential method; physiological relaxation; preventive medical effect

1. Introduction

More than half of the global population currently live in urban environments [1], and 66% of individuals are expected to live in urban areas by the year 2050 [2]. Although urbanization has led to improvements in many areas such as housing, employment, education, equality, quality of living environment, social support, and health services [3], changes occurring over a very short period have been very drastic from an evolutionary perspective. The gap between the highly urbanized and artificial environment that we modern humans currently inhabit, and our physiological functions which are best adapted to natural settings, is likely to contribute to a state of stress among people. Recent research has shown that city dwellers are constantly exposed to stressors and that urban living is associated with an increased risk of health problems [4–7].

As a result of such stressful situations in modern society, effective methods for coping with stress and for relaxation are receiving increasing attention. One such method is interaction with nature, because nature-based experiences are known to have a relaxing effect. Recent research has

demonstrated that the natural environment plays an important role in health promotion and that there is a positive relationship between nature-derived stimuli and human health [8–12].

In particular, there has been considerable and increasing attention in using the forest environment as a place for recreation and health promotion. This approach is called "Shinrin-yoku" in Japan and means "taking in the forest atmosphere through all of our senses" [13]. It suggests "forest bathing," which is a health promotion method that uses proven effects of a forest environment (such as relaxation) that can improve health of the body and mind. Based on the results of studies such as the above, the idea of "forest therapy" has been proposed. The evidence-based practice of "forest bathing (shinrin-yoku)" seeks to achieve preventive medical effects via the improvement of weakened immune functions and the prevention of diseases by achieving a state of physiological relaxation through exposure to forest-origin stimuli. Many studies have demonstrated the effects of walking in and/or viewing forests in mitigating stress states and inducing physiological relaxation [14–21]. Spending time in forests improves immune functions [22,23], and these effects last for approximately one month [24]. In addition, experiments with adults who are at risk of stress- and lifestyle-related diseases such as high blood pressure, diabetes, and depression, as well as elderly individuals, have found that various activities performed in forests have positive effects [25–32]. Restorative effects of forest environments on psychological stressors or mental fatigue, including decreased depressive symptoms and improved mood states, have been reported [18,20,33,34].

In addition, studies have been aimed at identifying elements of forests that bring about the above benefits. Forest-based stimuli are intuitively perceived through the five senses. Of the five senses, physiological effects of olfactory stimulation due to aromatic volatile substances derived from trees called phytoncides have been characterized in detail. The earliest experimental report in laboratory experiment, published in 1992 [35], showed that olfactory stimulation with Taiwan cypress essential oil significantly decreased blood pressure. Subsequently, other studies using Hinoki cypress (*Chamaecyparis obtusa*), a coniferous tree, have been conducted, clearly showing that inhalation of its leaf oil induced reduction in oxyhemoglobin concentrations in the prefrontal cortex and increased parasympathetic nervous activity, known to be associated with a relaxed state [36]. Wood oil from the above tree was found to increase natural killer cell activity and improve immune functions [37]. In addition, it has been reported that inhalation of α -pinene and D-limonene, which are major components of forest odor, decreased systolic blood pressure [38], enhanced parasympathetic nervous activity, and decreased heart rate [39,40].

However, most previous studies of physiological effects of forest-origin stimuli on humans have used olfactory stimulation, and examination of visual, auditory, and tactile stimuli have been limited. In contrast, recent developments in image projection technology have enabled visualization of images such as forests with greater clarity. Because visual stimulation derived from forests is expected to be familiar and convenient, we focused on visual stimulation. Ulrich et al. [41] investigated the effects of viewing a video about a natural environment on stress states, compared with effects of a video about an urban environment. Scenes of forests and streams were used to represent a natural environment. Measured parameters included skin conduction, muscle tension, and heart rate. The results showed that stress states were rapidly reduced upon exposure to the natural environment, compared with exposure to the urban environment. This was a pioneering study on the physiological effects of visual stimulation with forest imagery on humans; however, the study was limited by the fact that they only used indices of autonomic nervous activity as physiological responses.

In this study, we investigated the physiological effects of visual stimulation with forest imagery on left and right prefrontal cortex activity assessed using near-infrared time-resolved spectroscopy and on autonomic nervous activity assessed using heart rate variability.

2. Materials and Methods

2.1. Participants

Seventeen female Japanese university students (mean age \pm standard deviation: 21.1 ± 1.0 years) participated in the study. Mean height and weight were 158.2 ± 4.5 cm and 51.9 ± 6.2 kg, respectively. Participants who smoked, those currently in treatment for any disease, and those in their menstrual period during the study were excluded. All participants were informed about the aims and procedures of the study. After receiving a description of the experiment, they gave written consent to participate in the study. The study was conducted in accordance with guidelines of the Declaration of Helsinki, and the protocol was approved by the Ethics Committees of the Center for Environment, Health and Field Sciences, Chiba University, Japan (project identification no. 5).

2.2. Visual Stimulation

Visual stimulation was conducted using a 4K-compatible high vision liquid crystal display television of width 1872 mm and height 1053 mm and 3840 \times 2160 pixel resolution (85V type, TH-85AX900 by Panasonic, Osaka, Japan). A forest landscape showing Metasequoia (*Metasequoia glyptostroboides*) was used as forest imagery, and a scene of Shinjuku in Tokyo was used as control city imagery (Figure 1). The display was 1.4 m away from the participants.

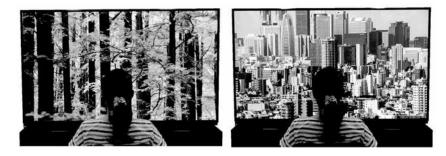


Figure 1. The scene during visual stimulation: (left) forest image; (right) city image.

2.3. Study Protocol

Physiological measurements were performed in a chamber with an artificial climate in the Center for Environment, Health and Field Sciences, Chiba University. This chamber was maintained at 24 °C, 50% relative humidity, and 50 lux illumination. The participants received a description of the experiment in a waiting room, and then moved into the chamber with an artificial climate. After sensors for physiological measurement were fitted, participants received a description of the measurement procedure while sitting. The study protocol is shown in Figure 2. After resting while viewing a gray background for 60 s (rest period), each participant was separately exposed to forest and city (control) images for 90 s each, while maintaining a sitting position. During the testing procedure, measurements of participants' physiological responses were continually performed. After finishing the 90 s visual stimulation, subjective evaluations were conducted. To eliminate any influences due to the order of viewing forest and city images, visual stimuli were presented in a counterbalanced order.

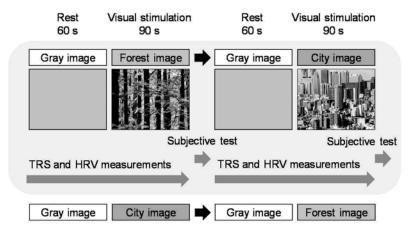


Figure 2. Study protocol. TRS: near-infrared time-resolved spectroscopy; HRV: heart rate variability.

2.4. Physiological Measurement

2.4.1. Near-Infrared Time-Resolved Spectroscopy (TRS)

As an indicator of brain activity, TRS, a near-infrared spectroscopy (NIRS)-based method, was used [42,43]. The sensors were mounted at approximately Fp1 and Fp2 of the international 10-20 system (electroencephalogram) on the participant's forehead (Figure 3), and oxyhemoglobin (oxy-Hb) concentration in the prefrontal cortex measured (TRS-20 system; Hamamatsu Photonics K.K., Shizuoka, Japan). Principles of the NIRS measurement are as follows. With an increase in local brain activity, brain blood flow increases and leads to significant perfusion such that quantity of brain blood flow exceeds oxygen consumption [44]. Consequently, oxy-Hb increases, and this increase can be detected. In addition, it is known that increases or decreases in quantity of blood flow in the brain are consistent with those of oxy-Hb [45], and it is thought that a decrease in oxy-Hb concentration causes physiological calming. The oxyhemoglobin (oxy-Hb) concentrations in the left and right prefrontal cortex were measured during the 60 s rest period and the 90 s visual stimulation period. Data measured using TRS-20 differ in sampling time. In the present experiment, data were measured at approximately 1.0–1.2 s intervals. We transformed the data using linear interpolation every second in order to show time series data of oxy-Hb concentration in the left and right prefrontal cortex. Each data point was calculated as the difference from the average of the 60 s rest period. Changes every 30 s and the overall mean during the 90 s visual stimulation period were analyzed.



Figure 3. Participant undergoing near-infrared time-resolved spectroscopy measurement.

2.4.2. Heart Rate Variability (HRV) and Heart Rate

As indicators of autonomic nervous activity, HRV and heart rate were used [46,47]. HRV was analyzed for the periods between consecutive R waves in the electrocardiogram (RR intervals) as measured by a portable electrocardiogram (Activtracer AC-301A; GMS, Tokyo, Japan). The power levels of the low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.40 Hz) components of HRV were calculated using the maximum-entropy method (MemCalc/Win; GMS, Tokyo, Japan) [48]. The HF power reflects parasympathetic nervous activity. The LF/HF ratio reflects sympathetic nervous activity. Changes in values of HF and LF/HF every 30 s period and overall mean during the 90 s of visual stimulation period were acquired, respectively. All data were calculated as differences from averages of the 60 s rest period.

2.5. Psychological Measurement

Psychological measurements were performed using the modified semantic differential (SD) method [49]. The SD method tests subjective evaluations of participants through a questionnaire with opposing adjectives, each of which was evaluated on a 13-point scale. Three pairs of adjectives were assessed; "comfortable–uncomfortable," "natural–artificial," and "relaxed–awakening."

2.6. Statistical Analysis

The software Statistical Package for Social Sciences (v21.0, IBM Corp., Armonk, NY, USA) was used for all statistical analyses.

Changes in physiological indices every 30 s were analyzed using paired *t*-tests with Holm correction to compare physiological responses between forest and city images; thus, the Holm correction [50,51] was applied three times. The Holm correction procedure is as follows. First, all *p*-values are sorted by size and then compared with increasing limits. The lowest limit is the overall limit divided by three, and the smallest *p*-value is to be compared with 0.05/3 or ca. 0.017. If the smallest *p*-value is >0.017, the process stops, but if it is smaller, the next smallest *p*-value is divided by two (p = 0.025). The process continues in a similar manner in that if the *p*-value is significant, the next smallest value is divided by one (p = 0.050). Overall mean values over the 90 s visual stimulation were analyzed using paired *t*-tests to compare physiological responses between the images.

Wilcoxon signed-rank test was used to analyze differences in psychological indices between the images.

One-sided tests were used for both comparisons, because we hypothesized that humans would be more relaxed after viewing forest imagery than city imagery.

3. Results

3.1. Physiological Effects

3.1.1. TRS

Figure 4 shows time-dependent oxy-Hb concentration change every second in the right prefrontal cortex during visual stimulation with forest or city images. Mean baseline of 60 s oxy-Hb concentration during the rest period did not significantly differ between the two stimuli (forest: $43.67 \pm 1.23 \mu$ M (mean \pm standard error), city: $43.58 \pm 1.22 \mu$ M; *p* > 0.05). However, oxy-Hb concentrations decreased immediately after viewing forest imagery and remained lower than the initial value throughout the rest of the experiment. Although oxy-Hb concentrations also decreased immediately after viewing city imagery, the value gradually increased during the course of the experiment.

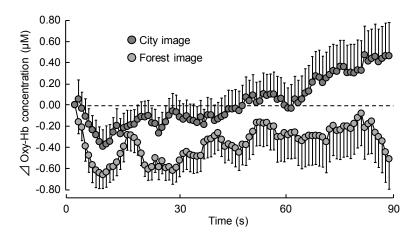


Figure 4. Change in oxyhemoglobin (oxy-Hb) concentration in the right prefrontal cortex during visual stimulation with forest and city images, every second over a 90 s period. Data are expressed as mean \pm standard error, *n* = 17.

Each 30 s average of oxy-Hb concentration in the right prefrontal cortex during visual stimulation with forest and city images is shown in Figure 5a. Mean oxy-Hb concentration in the 1–30 s period was $-0.50 \pm 0.09 \mu$ M during exposure to forest imagery, and $-0.19 \pm 0.13 \mu$ M during exposure to city imagery, showing a significant decrease (p < 0.05). Even in the 61–90 s period, it showed a significant decrease (p < 0.05) with mean concentration being $-0.26 \pm 0.26 \mu$ M during exposure to forest imagery and $0.27 \pm 0.24 \mu$ M during exposure to city imagery. However, there was no significant difference in mean oxy-Hb concentrations in the 31–60 s period (forest: $-0.33 \pm 0.16 \mu$ M; city: $-0.04 \pm 0.16 \mu$ M).

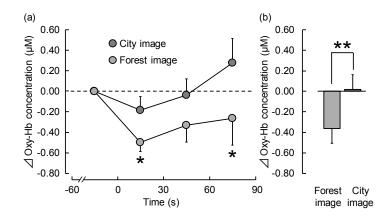


Figure 5. Thirty-second average and overall mean oxyhemoglobin (oxy-Hb) concentration in the right prefrontal cortex during visual stimulation with forest and city images. (a) Changes in each 30 s average oxy-Hb concentration over 90 s. (b) Overall mean oxy-Hb concentrations. Data are expressed as means \pm standard errors, n = 17, * p < 0.05, ** p < 0.01, as determined by the paired *t*-test (one-sided); the Holm correction was applied.

Figure 5b shows overall mean oxyhemoglobin (oxy-Hb) concentration in the right prefrontal cortex during visual stimulation with forest and city images. Viewing forest imagery significantly increased oxy-Hb concentration compared with viewing city imagery (forest: $-0.36 \pm 0.14 \mu$ M; city: $0.02 \pm 0.14 \mu$ M; *p* < 0.05).

However, there was no significant difference in the left prefrontal cortex (forest: $-0.20 \pm 0.16 \mu$ M; city: $0.05 \pm 0.18 \mu$ M; *p* > 0.05).

3.1.2. HRV and Heart Rate

There were no significant differences in the HF value, which is an index of parasympathetic nervous activity, LF/HF ratio, which is an index of sympathetic nervous activity, and heart rate between participants viewing forest and city images.

3.2. Psychological Effects

Figure 6 shows the results of subjective feelings as measured by a modified SD questionnaire after visual stimulation with forest and city images. Participants felt more "comfortable," "natural," and "relaxed" when they viewed forest imagery, compared with city imagery (p < 0.01).

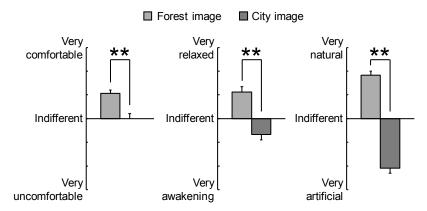


Figure 6. Subjective feelings measured with the modified semantic differential method after viewing forest and city images. Data are expressed as means \pm standard errors, n = 17, ** p < 0.01 as determined by the Wilcoxon signed-rank test.

4. Discussion

This study examined the physiological effects of visual stimulation with forest imagery on left and right prefrontal cortex activity assessed using TRS and on autonomic nervous activity assessed using HRV. In addition, subjective assessments of psychological relaxation were conducted. Results of physiological measurements showed that viewing forest imagery significantly decreased oxy-Hb concentrations in the right prefrontal cortex, compared with viewing city imagery. Table 1 shows the results of physiological and psychological measurements.

Table 1.	Physiological	and psycl	hological	measurements.

	Forest Image	City Image	<i>p</i> -Value	Significance Level
Physiological Measurement $(n = 17)$	$Mean \pm SE$	Mean \pm SE		
Wiedsurement $(n - 17)$	μΜ	μΜ	_	
Oxy-Hb concentration in the				
right prefrontal cortex				
1–30 s	-0.50 ± 0.09	-0.19 ± 0.13	0.024	* $p < 0.05$
31–60 s	-0.33 ± 0.16	-0.04 ± 0.16	0.097	NS
61–90 s	-0.26 ± 0.26	0.27 ± 0.24	0.011	* $p < 0.05$
Overall mean	-0.36 ± 0.14	0.02 ± 0.14	0.006	** <i>p</i> < 0.01
Oxy-Hb concentration in the				
left prefrontal cortex				
1–30 s	-0.31 ± 0.09	-0.13 ± 0.14	0.131	NS
31–60 s	-0.26 ± 0.16	-0.10 ± 0.18	0.260	NS
61–90 s	-0.02 ± 0.27	0.37 ± 0.30	0.087	NS
Overall mean	-0.20 ± 0.16	0.05 ± 0.18	0.112	NS

Psychological Measurement ($n = 17$)	Forest Image Mean ± SE Score	City Image Mean ± SE Score	pValue	Significance Level
"Comfortable" feeling "Relaxed" feeling "Natural" feeling	$2.1 \pm 0.3 \\ 2.2 \pm 0.4 \\ 3.6 \pm 0.3$	$0.0 \pm 0.4 \\ -1.4 \pm 0.4 \\ -4.2 \pm 0.4$	<0.001 <0.001 <0.001	** <i>p</i> < 0.01 ** <i>p</i> < 0.01 ** <i>p</i> < 0.01

Table 1. Cont.

* *p* < 0.05, ** *p* < 0.01, NS: not significant.

Assessing the effects of spending time in a forest environment on brain activity, Park et al. [14] reported that walking for 15 min in a forest area reduced total hemoglobin concentration in the left prefrontal cortex, compared with walking in a city area. Joung et al. [52] found that viewing a forest landscape, compared to viewing an urban landscape, from the roof of an urban building for 15 min decreased total hemoglobin concentration and oxy-hemoglobin concentration in the prefrontal cortex. Testing the effect of olfactory stimulation by forest-origin stimuli on brain activity, Ikei et al. [36] demonstrated that inhalation of Hinoki cypress leaf oil reduced oxyhemoglobin concentrations in the right prefrontal cortex. However, studies of the effects of forest stimuli on brain activity are limited. The results of this study show a significant decrease in oxy-Hb concentrations in the right prefrontal cortex, partly consistent with findings of previous studies in the context of calming brain activity. However, reason(s) for no significant difference in the left prefrontal cortex is unknown; it is necessary to obtain more data on the influence of forest-derived stimuli on prefrontal cortex activity to clarify any differences in response between left and right prefrontal cortices. Autonomic nervous activity (heart rate, sympathetic nervous activity, and parasympathetic nervous activity) did not change significantly. Kahn et al. [53] also investigated the physiological effect of plasma display windows using heart rate. In an office setting, 90 participants (30 per group) were exposed either to (a) a glass window that afforded a view of a nature scene, (b) a plasma window that afforded a real-time HDTV view of essentially the same scene, or (c) a blank wall. Results showed that in terms of heart rate recovery from low-level stress, the glass window was more restorative than a blank wall. However, the plasma window was no different from the blank wall. A similar result was obtained with respect to heart rate. These findings need to be confirmed using different experimental design settings and visual stimulation methods in future studies.

In addition, results of subjective assessments of psychological relaxation showed that viewing forest imagery significantly increased perceptions of feeling "comfortable," "relaxed," and "natural" compared with viewing city imagery. These psychological benefits of viewing forest imagery can be considered significant in modern times, given that mental health problems associated with living in urban environments are profound [5,7]. Further, application of these findings is expected to play an important role in improvement of psychological stress states in the future.

The findings of this study provide rational scientific evidence for beneficial physiological and psychological effects of viewing forest imagery in young women in their 20s. To generalize these findings, further studies based on a larger sample including other demographic groups such as males and different age groups, are required. Further, it is necessary to examine such effects not only in healthy individuals but also in populations who experience a heightened state of stress in daily life. In addition, in future studies, it will be necessary to elucidate the effect of differences in magnification, angles, and display hue between forest and city imagery.

5. Conclusions

The findings of this study provide significant scientific evidence of the physiological effects of visual stimulation with forest imagery on brain activity and autonomic nervous activity. Viewing forest imagery induced (1) a significant decrease in oxy-Hb concentrations in the right prefrontal cortex

and (2) a significant increase in perceptions of feeling "comfortable," "relaxed," and "natural." These findings indicate that viewing forest imagery may induce physiological and psychological relaxation.

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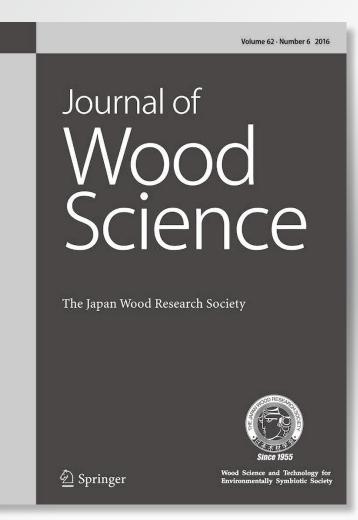
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RAPID COMMUNICATION



Effects of olfactory stimulation by α -pinene on autonomic nervous activity

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Keywords α -Pinene \cdot Smell \cdot Physiological relaxation \cdot Heart rate variability \cdot Semantic differential method

Introduction

Wood has been used as building material and for making furniture since a long time, and it has been known from experience that woody smell acts as a mood relaxant. Several studies on the physiological and psychological effects of wood or wood-derived smells have been conducted [1–7]. The inhalation of air containing volatile organic compounds released from the interior walls of Japanese cedar suppresses increases in salivary chromogranin A [1]. Olfactory stimulation by Japanese cedar chips decreases systolic blood pressure and prefrontal cortex activity [2]. Olfactory stimulation by air-dried wood chips of Japanese cypress, which is commonly found and is widely used as a building material in Japan, reduced the oxygenated hemoglobin concentration in the prefrontal cortex [3]. Moreover, it has been reported that staying at

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night in a hotel room filled with the smell of Japanese cypress essential oil for three consecutive nights induces natural killer cell activity and reduces the concentrations of adrenaline and noradrenaline in urine [4]. Olfactory stimulation by the essential oil from Japanese cypress leaf enhances parasympathetic nervous activity and decreases prefrontal cortex activity; in a subjective evaluation, the stimulation was assessed to be "comfortable" [5]. Inhalation of D-limonene, which is a major component of conifer wood extracts such as Japanese cedar and Japanese cypress, enhanced parasympathetic nervous activity and decreased heart rate; in a subjective evaluation, the stimulation was also assessed to be "comfortable" [6]. Inhalation of cedrol, a compound found in cedar extract, induced parasympathetic nervous activity and reduced sympathetic nervous activity [7].

 α -Pinene is a typical volatile compound present in Japanese cedar wood [8], which is used as a general architectural material. It is also the main component responsible for the smell in forests [9]. Studies using rats or mice have reported the physiological effects of α -pinene in rodent species [10, 11]. In human studies, Tsunetsugu et al. [12] investigated the effects of α -pinene on 15 male college students. They found that olfactory stimulation with α -pinene, which was rated as a "slight smell", decreased systolic blood pressure and was assessed as "slightly comfortable" in the subjective evaluation [12]. However, no study has evaluated the physiological effects of α -pinene inhalation on adult females using heart rate variability (HRV) as an index.

In this study, we investigated the effects of olfactory stimulation by α -pinene on autonomic nervous activity based on the assessment of parasympathetic nervous activity and sympathetic nervous activity using HRV and heart rate in young adult females.

Materials and methods

Thirteen Japanese young adult females were recruited. In addition, none of the participants were menstruating on the day of the experiment. The participants who participated in the study had a mean age (\pm standard deviation) of 21.5 \pm 1.0 years. All participants were informed about the aims and procedures involved in the experiment provided their written informed consent for participation. This study was performed in accordance with the regulations of the Ethics Committee of the Center for Environment, Health and Field Sciences, Chiba University, Japan.

Physiological measurements of the participants were performed in a chamber with an artificial climate maintained at 25 °C, 50 % relative humidity, and 230 lx illumination. After fitting the sensors for the physiological measurements, participants received a description of the measurement procedure again for 10 min while sitting. The participants then rested by sitting with their eyes closed, and the smell was administered for 90 s; subsequently, the subjective evaluation test was performed. A crossover trial to eliminate any effects due to the order of olfactory stimulation was performed. Approximately half the participants were administered stimuli in the following order: exposure to α -pinene followed by control (air). The remaining participants were presented with the control followed by α -pinene.

 α -Pinene (Tokyo Chemical Industry Co., Ltd., Japan) was used as the olfactory stimulant, and air was used as the control. To administer the stimulation, α -pinene (20 µL) was injected into a smell bag (polyethylene terephthalate film heat seal bag; NS-KOKEN Co., Ltd., Japan) filled with 24 L air. After vaporizing the α -pinene in the smell bag using a dryer, the smell bag was incubated for approximately 1 h at room temperature to diffuse the α -pinene into the bag. Smells were administered to each participant by means of a device fixed on the chest and situated approximately 10 cm under the nose (Fig. 1). The flow rate of the air saturated with α -pinene was 3 L/min. Preliminary investigations determined that the subjective intensity of the smell was "weak" or "easily sensed".

As an indicator of physiological condition, HRV was analyzed using the periods between consecutive R waves (R–R intervals) on electrocardiograms measured using a portable electrocardiograph (Activtracer AC-301A; GMS, Japan) [13, 14]. This device performs measurements using a 3-lead electrocardiogram (Lead II). The power levels of the low-frequency (LF: 0.04–0.15 Hz) and high-frequency (HF: 0.15–0.40 Hz) components of HRV were calculated using the maximum entropy method (MemCalc/Win; GMS, Japan) [15]. The HF power reflects parasympathetic nervous activity, which increases in the relaxed state. The LF/(LF + HF) ratio reflects sympathetic nervous activity, which increases in the arousal or stressed state. Data were

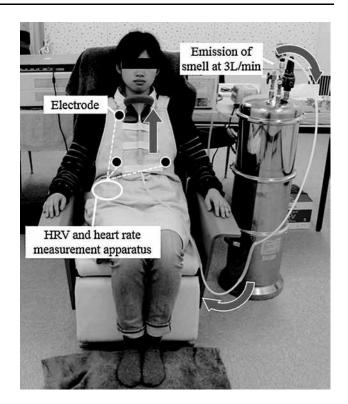


Fig. 1 Olfactory stimulation setup

acquired for 30 s before smell administration and during the 90-s smell administration. Heart rate was also investigated using R-R interval data.

To subjectively evaluate the psychological effect of the smell, the participants were tested using the modified semantic differential (SD) method [16]. Three pairs of adjectives were assessed on 13 scales as "comfortable–uncomfortable", "relaxed–awakening", and "natural–artificial".

All data are shown as the mean \pm standard error. Physiological and psychological tests were used to compare α -pinene with the control. All statistical analyses were performed using Statistical Package for Social Sciences version 20.0 software (IBM Corp., Armonk, NY, USA). A paired *t* test was used to compare the physiological responses to α -pinene with those to the control. The Wilcoxon signed-rank test was used to analyze differences in psychological indices between the responses to the α -pinene and those to the control. A one-sided test was used in this study because of the hypothesis that humans would be relaxed on inhaling α -pinene. In all cases, the significance level was set at *P* < 0.05.

Results and discussion

The HF value associated with olfactory stimulation by α pinene is shown in Fig. 2a. The mean baseline HF for 30 s before stimulation (pre-measurement condition) did not

differ significantly between the α -pinene group $(760.0 \pm 249.7 \text{ ms}^2)$ and control group $(793.2 \pm$ 287.2 ms²). Figure 2b shows the overall mean of the HF value associated with olfactory stimulation by α -pinene. When the results of the HRV power level data were compared, a significant difference was found in the HF power level between the α -pinene and control groups (P < 0.05).The HF power level of α -pinene $(967.3 \pm 192.3 \text{ ms}^2)$ was 46.8 % higher than that of the control group $(658.7 \pm 161.8 \text{ ms}^2)$. It was clear that olfactory stimulation by the α -pinene induced a significant increase in parasympathetic nervous activity and thereby induced physiological relaxation. However, no significant difference was found in the LF/(LF + HF) ratio between groups receiving the two stimuli (α -pinene, 0.30 \pm 0.05; control, 0.35 ± 0.06).

Figure 3 shows the heart rates measured during olfactory stimulation by α -pinene or control. The mean baseline heart rate at 30 s before stimulation (premeasurement condition) did not differ significantly between the α -pinene group $(73.3 \pm 2.4 \text{ beats/min})$ and the control group $(74.5 \pm 2.6 \text{ beats/min})$, which is similar to the results observed with regard to the HF component. The mean heart rate during olfactory stimulation by α -pinene remained lower than that of the control and gradually decreased from the baseline (Fig. 3a). A comparison of the mean heart rates of 90-s olfactory stimulation by α -pinene and control is shown in Fig. 3b. Olfactory stimulation by α -pinene significantly decreased the heart rate compared with control (Fig. 3b, P < 0.05). The heart rate of α -pinene group $(72.0 \pm 2.3 \text{ beats/min})$ was 2.8 % lower than that of the control group (74.1 \pm 2.6 beats/min).

The modified SD method was used to provide subjective reports of "comfortable", "relaxed", and "natural" feelings (Fig. 4). When subjected to the stimulation by α -pinene, participants provided subjective reports of feeling

"slightly comfortable"; however, they provided reports of feeling "indifferent" when subjected to the control. Therefore, the response to α -pinene was perceived as being significantly more comfortable than that to the control (Fig. 4 left, P < 0.05). Although the differences were not statistically significant, the results suggest that α -pinene was more "relaxed" and "natural" than the control (Fig. 4 center, P = 0.077 and right, P = 0.097).

This study was designed to clarify the effects of olfactory stimulation by α -pinene on autonomic nervous activity. The effects were assessed by measuring HRV and heart rates of young adult females. The results showed that olfactory stimulation with α -pinene significantly increased parasympathetic nervous activity and significantly decreased heart rate.

Our previous studies of HRV demonstrated significant differences in parasympathetic nervous activity but not in sympathetic nervous activity. Olfactory stimulation by Japanese cypress leaf oil and inhalation of D-limonene enhanced parasympathetic nervous activity by 34.5 and 26.4 %, respectively, compared with a control (air) [5, 6]; these findings were in accordance with the results of our previous laboratory experiments [17, 18]. In our forest therapy field experiment, which included a large sample size of 625 participants [19], 79.2 % of the participants showed an increase in parasympathetic nervous activity in a forest environment compared with that in an urban environment. However, only 63.5 % of the participants exhibited decreases in sympathetic nervous activity [19]. Based on these findings, we concluded that the parasympathetic nervous activity index of HRV was more sensitive than the sympathetic nervous activity index.

Subjective evaluations demonstrate that the participants felt more comfortable after olfactory stimulation by α -pinene than by the control. Olfactory stimulation by α -pinene, which were rated as "slight smell", was assessed to

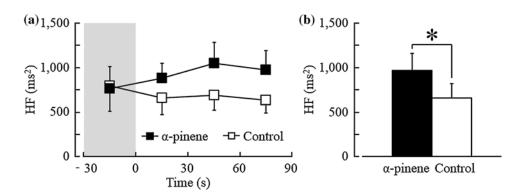
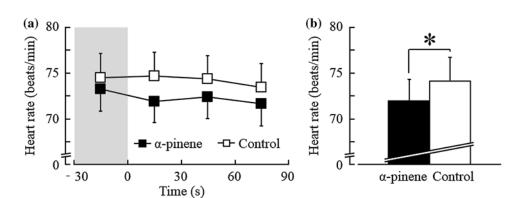


Fig. 2 The 30-s means and overall mean high-frequency (HF) component of heart rate variability (HRV) during olfactory stimulation by α -pinene or control. **a** Changes in each 30-s mean HF value

over 90 s. **b** Overall mean HF values. Data are expressed as the mean \pm standard error; N = 13; *P < 0.05 as determined using the paired *t* test (*one sided*)

Deringer

Fig. 3 The 30-s means and overall mean heart rate during olfactory stimulation by α pinene or control. **a** Changes in each 30-s mean heart rate over 90 s. **b** Overall mean heart rate. Data are expressed as the mean \pm standard error, N = 13, *P < 0.05 as determined using the paired t test (one sided)



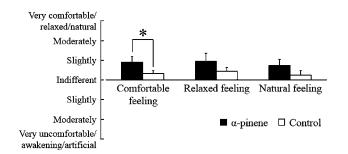


Fig. 4 Subjective feelings measured by the modified semantic differential method after olfactory stimulation by α -pinene or control. Data are expressed as the mean \pm standard error, N = 13, *P < 0.05 as determined by the Wilcoxon signed-rank test (*one sided*)

be "slightly comfortable" in the subjective evaluation [12]; this finding is consistent with the results of this study. The results of this study also match those of previous studies, including studies of D-limonene from wood-derived components [6] and Japanese cypress leaf oil [5].

Wood is a familiar natural material because it is globally used as a building material or for making furniture. In recent years, the accumulation of data on the physiological effects of wood or wood-derived stimuli, such as smell [1–7, 12], viewing [20–22], and touch [23], has been promoted. In this study, we clarified the physiological relaxation effects of olfactory stimulation by α -pinene. In the future, it is possible that accumulating scientific evidence about wood-derived smells and clarifying the physiological relaxation effects on individuals living in areas in which substantial quantities of wood are present will help improve the quality of life of modern people.

Although this study evaluated autonomic nervous activity, other experimental indices such as brain activity, which can be measured using near-infrared spectroscopy, and stress hormone levels, which can be measured using salivary cortisol concentration, should be assessed to more comprehensively evaluate the physiological effects of olfactory stimulation by α -pinene. In addition, the participants of this study were young adult females. Studies on males, minors, and elderly are required.

Conclusions

Olfactory stimulation by α -pinene significantly increased the HF component of HRV, which is associated with parasympathetic nervous activity, and significantly decreased heart rate. These findings indicate that olfactory stimulation by α -pinene induces physiological relaxation.

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Physiological Effects of Nature Therapy: A Review of the Research in Japan

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Abstract: Humans have evolved into what they are today after the passage of 6–7 million years. If we define the beginning of urbanization as the rise of the industrial revolution, less than 0.01% of our species' history has been spent in modern surroundings. Humans have spent over 99.99% of their time living in the natural environment. The gap between the natural setting, for which our physiological functions are adapted, and the highly urbanized and artificial setting that we inhabit is a contributing cause of the "stress state" in modern people. In recent years, scientific evidence supporting the physiological effects of relaxation caused by natural stimuli has accumulated. This review aimed to objectively demonstrate the physiological effects of nature therapy. We have reviewed research in Japan related to the following: (1) the physiological effects of nature therapy, including those of forests, urban green space, plants, and wooden material and (2) the analyses of individual differences that arise therein. The search was conducted in the PubMed database using various keywords. We applied our inclusion/exclusion criteria and reviewed 52 articles. Scientific data assessing physiological indicators, such as brain activity, autonomic nervous activity, endocrine activity, and immune activity, are accumulating from field and laboratory experiments. We believe that nature therapy will play an increasingly important role in preventive medicine in the future.

Keywords: natural environment; shinrin-yoku; forest bathing; urban green space; plant; wooden material; physiological relaxation; evidence-based medicine (EBM); preventive medicine; individual difference

1. Introduction

Despite living in this modern era and surroundings, our bodies are best adapted to living in a natural environment [1]. This could be because 6–7 million years ago our ancestors started evolving from a subset of primates into our current form [2], and early humans spent over 99.99% of that time living in a natural environment. If we define the beginning of urbanization as the rise of the industrial revolution, less than 0.01% of our species' history has been spent in modern surroundings. The gap between natural settings, to which our physiological functions are best adapted, and the highly urbanized and artificial environment that we inhabit is a contributing cause of the "stress state" in modern people. In addition, the emergence of "megacities", such as Tokyo and New York, which house more than 10 million inhabitants, has had an enormous influence on human lifestyles [3,4].

Rapid changes have also occurred in our environment over the last three decades, including the widespread use of computers. In 1984, American clinical psychologist Craig Brod coined the term "technostress" [5]. Other forms of technologies that expose us to more artificial elements have also contributed to the exacerbation of our stress levels.

As a result of these stressful situations, nature therapy, a health-promotion method that uses medically proven effects, such as relaxation by exposure to natural stimuli from forests, urban green spaces, plants, and natural wooden materials, is receiving increasing attention. It is empirically known that exposure to stimuli from natural sources induces a state of hyperawareness and hyperactivity of the parasympathetic nervous system that renders a person in a state of relaxation. This state becomes progressively recognized as the normal state that a person should be in and feel comfortable. Many conventional studies [6–11] have pointed out the benefits of nature therapy, but there is a lack of data that can shed light on these benefits on the body using evidence-based medicine (EBM), which is conventionally focused on physiological indications such as brain activity, autonomic nervous system activity, endocrine activity, and immune activity. Recently, the submission of scientific data on the basis of EBM was socially requested. In this review, we introduce the current situation on data accumulation.

Nature therapy is defined as "a set of practices aimed at achieving 'preventive medical effects' through exposure to natural stimuli that render a state of physiological relaxation and boost the weakened immune functions to prevent diseases" [12]. Unlike "specific effects" that are typically anticipated from pharmacological treatments, nature therapy seeks to improve immune functions, prevent illnesses, and maintain and promote health through exposure to nature, with the consequent attainment of a state of relaxation (Figure 1) [12,13].

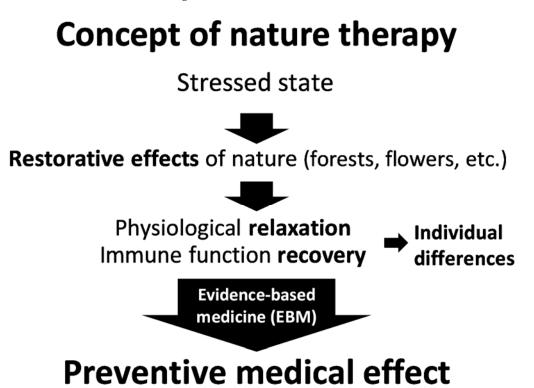


Figure 1. Concept of nature therapy [12].

In 1990, the first investigation on the physiological effects of being in a forest environment was performed in Japan [14–16], and active research has been conducted since. Therefore, this review aims to summarize research in Japan with regard to the following points: (1) activities in the central nervous system, autonomic nervous system, endocrine system, and immune system as functions of physiological relaxation as well as the immunity recovery effects of forest, urban green space, plants, and wooden material therapies along with (2) the analyses of individual differences that arise therein.

2. Methods

A scientific review of the accessible literature published over the last 20 years was conducted. Inclusion criteria for studies in this review were (1) studies conducted in Japan; (2) publication in the English language; and (3) publication from January 1996 to June 2016. Exclusion criteria for studies in this review were (1) review articles and (2) articles with only an abstract available. The search for relevant papers was conducted in the PubMed database with the keywords: "forest therapy", "forest bathing", "shinrin-yoku", "urban park", "urban green space", "plant therapy", "aroma therapy", "horticultural therapy", "foliage plants", "fresh flowers", "natural wooden material", "Hinoki cypress", "Japanese cypress", "Japanese cedar", and "physiological relaxation". One thousand three-hundred and eight references were identified from this search. In addition, other publications cited in the collected papers were examined and added to the relevant literature. We applied our inclusion/exclusion criteria and retained 52 articles for our review.

Moreover, research related to the stimulation effects from nature in Japan includes forest, urban green space, plants (flowers and foliage plants), and wooden material effects, which we included in our study. The definition of each therapy (forest therapy, urban green space therapy, plant therapy, and wooden material therapy) indicates the therapeutic effect brought about by each of the stimulus.

3. Physiological Effects of Nature Therapy

3.1. Forest Therapy

In recent years, there has been considerable and increasing attention in using the forest environment as a place for recreation and health promotion. This approach is called "Shinrin-yoku" in Japan and means "taking in the forest atmosphere through all of our senses" [14]. It suggests "forest bathing", which is a health promotion method that uses proven effects of a forest environment, such as relaxation, that can improve the health of the body and mind. In accordance with the accumulation of data, the idea of "forest therapy" has been proposed. Evidence-based "forest bathing (shinrin-yoku)" seeks preventive medical effects to improve weakened immune functions and prevent diseases by achieving a state of physiological relaxation through exposure to forest-origin stimuli.

In 1990, a preliminary study aimed to investigate the physiological effect of "Shinrin-yoku" using salivary cortisol levels, a marker of stress hormone [14–16]. Although the sample size was small, the result indicated that spending time in a forest environment can reduce stress state [16]. From 2005 to 2015, a physiological experiment was conducted over a one-week period on 744 participants in 62 forests located all over Japan. There are many reports related to this experiment describing indicators such as salivary cortisol levels [17–24], heart rate variability(HRV)-related sympathetic [22–27] and parasympathetic nervous activity [20–27], blood pressure [19,21–23,27], and pulse rate [19–24,27] to demonstrate the effects of relaxation.

In one particular study, the results on 280 participants (average age: 21.7 years) in 24 locations were reported [22]. The forest therapy experiment was conducted in forests representing the environmental characteristics of each region. The control experiment was conducted in an urban setting, such as stations and urban centers in large cities of the respective prefectures, following the same experimental schedule. The participants were 12 male university students who were residents of each region included in this experiment. The participants were divided into two groups of six persons each. On the first day, one group visited in the forest environment, whereas the other group visited in the urban environment; on the second day, the groups were switched. Upon arrival at the given site, the participants sat in chairs viewing the landscapes of their assigned areas. They also walked around their assigned areas. These activities were performed for about 15 min. Salivary cortisol levels, systolic and diastolic blood pressure, pulse rate, and HRV were measured. Saliva was collected utilizing a saliva collection tube, and cortisol was analyzed after storage in refrigerated and frozen environments (SRL Inc., Tokyo, Japan). Pulse rate and blood pressure were measured from the upper right arm using a digital sphygmomanometer based on the oscillometric method (HEM-1000, Omron, Tokyo, Japan). HRV was

analyzed using the maximum entropy method (MemCalc/Win, GMS, Tokyo, Japan) after measuring the R-R interval (Activtracer AC-301A, GMS, Tokyo, Japan), setting the low-frequency component (LF) at 0.04–0.15 Hz and the high-frequency component (HF) at 0.15–0.40 Hz. The results indicated that the salivary cortisol level of participants was 13.4% lower after viewing the forest area compared to after viewing the urban area. The pulse rate and systolic and diastolic blood pressures were lower by 6.0%, 1.7%, and 1.6%, respectively. HF, which is known to reflect parasympathetic nervous activity, increased by 56.1% during forest therapy, and the LF/HF ratio, which is known to reflect sympathetic nervous activity, decreased by 18.0%. For the walking experiment, salivary cortisol levels were 15.8% lower after walking in the forest area compared to walking in the urban area. The pulse rate and systolic and diastolic blood pressures were lower by 3.9%, 1.9%, and 2.1%, respectively. HF increased by 102.0% and the LF/HF ratio decreased by 19.4%. The results after walking were very similar to those obtained in the seated position.

Based on these results, it can be concluded that forest therapy had the following effects: (1) it decreased the levels of salivary cortisol, a typical stress hormone; (2) it decreased the pulse rate; (3) it decreased the systolic and diastolic blood pressures; (4) it increased HF; and (5) it decreased the LF/HF ratio. These findings show that viewing or walking around a forest environment for a 15 min session of forest therapy induces a state of physiological relaxation. The results were almost identical to those of an experiment conducted on 420 participants in 35 locations [23].

In 2007, prefrontal cortex activity was measured using near-infrared time-resolved spectroscopy (TRS) [18]. Absolute hemoglobin concentrations in the prefrontal cortex were measured (TRS-10; Hamamatsu Photonics K.K., Hamamatsu, Japan). The total hemoglobin concentration was lower after walking in a forest area than in a city area.

In contrast to most previous studies that experimented on healthy participants in their 20s, a study evaluated the effects of a 17 min forest walk on hypertensive middle-aged participants using HRV and heart rate as indicators [28]. Twenty hypertensive males (average age: 58.0 years; systolic blood pressure: 151.2 mmHg; diastolic blood pressure: 90.7 mmHg) participated. They walked in a coniferous forest that included many Japanese cypress trees (Akasawa natural recreation forest) and a corresponding urban area. The results showed that walking through the forest decreased heart rate and increased ln(HF) in comparison with walking in the urban area.

Next, nine hypertensive males (average age: 56.0 years) were studied to demonstrate the effects of a forest therapy program starting at 10:30 and ending at 15:05 [29]. Participants walked around their assigned area and then sat down and lay on their backs in the forest on waterproof sheets laid on the ground. This experiment was also conducted in a forest in Akasawa natural recreation forest, and physiological measurements were taken between 15:14 and 15:35. The measurements were taken at the same time on the day prior as a control to compare the experimental results with measures taken on a normal day. The comparison showed that forest therapy decreased the systolic blood pressure to 123.9 mmHg from the control value of 140.1 mmHg, and it decreased the diastolic blood pressure to 76.6 mmHg from the control value of 84.4 mmHg. Moreover, reductions in urine adrenaline and serum cortisol levels were also noted. These observations evidenced the physiological relaxation effects of a forest therapy program lasting a few hours on hypertensive male participants.

Furthermore, 17 middle-aged females (average age: 62.2 years) participated in a study with the same experimental design and locations the following year [30]. Similar results were obtained. Forest therapy elicited a decrease in pulse rate and salivary cortisol levels. There were substantial physiological benefits of forest therapy in middle-aged females.

Studies about patients with non-insulin-dependent diabetes also have been performed [31]. A total of 87 non-insulin-dependent diabetic patients (29 male and 58 female; average age: 61 years) walked in the forest nine times over a period of six years. Blood glucose levels of patients decreased after forest walking on all nine occasions. The mean blood glucose level showed a reduction from $179 \text{ mg} \times 100 \text{ mL}^{-1}$ to $108 \text{ mg} \times 100 \text{ mL}^{-1}$ after walking in the forest. This indicates that forest therapy has beneficial effects on blood glucose levels.

Three studies by Li et al. [32–34] demonstrated a boosting effect of forest therapy on weakened immune functions. Twelve male company employees aged between 37 and 55 years with weakened immune function were studied to observe the effects on natural killer (NK) cell activity. The forest therapy lasted two nights and three days [32]. The initial NK-cell activity value was measured at 8 a.m. upon arriving at the work environment three days before participation in the forest therapy. The participants traveled to the forest in the morning of Day 1 and took a walk in the forest for approximately 2 h in the afternoon, over a distance of 2.5 km. Blood samples were drawn at 8 a.m. on Day 2 as data for Day 1. The participants took a 2 h, 2.5 km walk in the morning and afternoon of Day 2. Again, blood samples were collected at 8 a.m. on Day 3 as data for Day 2. After the forest therapy, NK-cell activity had increased by 1.25 times on Day 1 and by 1.5 times on Day 2. Thus, this finding confirmed the immunity-boosting effects of forest therapy on NK cell activity. Another experiment with a similar design was conducted in the following year on male employees with weakened NK cell activity, and the results showed almost identical effects [33]. A similar two-night, three-day forest therapy experiment conducted on female nurses aged between 25 and 43 years also showed improvements in weakened NK cell activity, thereby demonstrating the similar effectiveness of this therapy in male and female participants [34].

Li et al. also studied the long-term effects of forest therapy [33,34]. Measures were taken at one week and one month after the male and female participants of the abovementioned experiments returned to work. High NK cell activity levels were maintained in both male and female groups at one week, and they were found to be maintained in the male group at one month.

To eliminate the effect of environmental change as a possible confounding factor, participants had a vacation of the same length of time to an urban area to test the effects of simple environmental change on NK cell activity [33]. The participants were males who underwent forest therapy, and the program involving the same hours and distance of walking as in the forest therapy group was replicated. The results of this experiment showed no improvement in weakened NK cell activity during the stay in the urban setting on Days 1 or 2.

From the above, the following effects of forest therapy were determined: (1) it improves weakened NK cell activity in male participants; (2) it shows the same effects in female participants; (3) these effects last in male group for a month; and (4) none of these improvements are observed in a population exposed to an urban setting.

In addition, several indoor studies focused on forest-derived olfactory stimuli exist. Hinoki cypress (*Chamaecyparis obtusa*), a coniferous tree, is a common and familiar tree in Japan. The effects of indoor exposure to Hinoki cypress wood oils on NK cell activity were examined [35]. The participants were 12 male instructors aged between 37 and 60 years who worked at a university. The experiment consisted of a three-night four-day stay in a metropolitan Tokyo hotel. The results showed that NK cell activity was increased and immune functions had improved. Therefore, olfactory stimulation brought about improvements in immune functions.

To investigate the effects of its leaf oil on brain activity and autonomic nervous activity, the experiment was conducted in an artificial climate chamber with the temperature, humidity, and illuminance set at 25 °C, 50%, and 230 lx, respectively [36]. A total of 13 female university students (average age: 21.5 years) were exposed to Hinoki cypress leaf oils, whereas a control group was not exposed to any odors (air). The odor was administered for 90 s, while participants sat with their eyes closed. Olfactory stimulation by Hinoki cypress leaf oil induced a reduction in oxyhemoglobin concentrations in the prefrontal cortex and increased parasympathetic nervous activity. Therefore, olfactory stimulation by Hinoki cypress leaf oil can induce physiological relaxation.

3.2. Urban Green Space Therapy

The effects of urban green space are now attracting attention as another source of nature in an accessible form. Recent demographic studies have found a positive association between exposure to urban green space and the perceived general health of residents [37]. Living in areas with accessible

green spaces for walking also increases the longevity of senior citizens, independent of age, sex, marital status, baseline functional status, and socioeconomic status.

In a previous study [38], participants were asked to walk in an urban park during the spring season, and the control group was asked to walk in a street (hereinafter, urban setting) near the urban park. The temperature and humidity levels in the urban park were 24.7 °C and 39%, respectively, compared with 27.0 °C and 37% in the urban setting. The physiological indicators were HRV and heart rate, and the participants were 17 male university students (average age: 21.2 years). The participants were separated into groups of two for counterbalancing, and they walked in their respective environments for 15 min. The results showed that walking in the park increased ln(HF) and decreased the ln(LF/HF) ratio compared with walking in the urban setting. Decreased heart rates were also measured in the group that walked in the urban park. In conclusion, walking in an urban park in the spring (1) increases parasympathetic nervous activity; (2) inhibits sympathetic nervous activity; and (3) decreases the heart rate, thereby showing physiological relaxation effects.

The same experiment was performed in the fall (autumn) season [39]. The temperature and humidity levels in the urban park were 18.0 °C and 72%, respectively, compared with 19.2 °C and 65% in the urban setting. Twenty-three male university students (average age: 22.3 years) walked in an urban park and city area for 15 min. A brief walk in an urban park during fall induced parasympathetic nervous activity, suppressed sympathetic nervous activity, and decreased the heart rate.

The experiment was also conducted in the winter season [40] (temperature: 13.8 °C, humidity: 51%). Thirteen male university students (average age: 22.5 years), wearing protective gear against the cold with a hat, gloves, and other winter accessories, were asked to walk in the park. The results showed that the group that walked in the park showed an increase in ln(HF) and a decrease in the heart rate. From this, we can conclude that walking in a park has a physiological relaxation effect even in the winter.

Recently, urban gardening has also been attracting attention as another source of accessible exposure to nature. To study its effects, the physiological relaxation effects of viewing a kiwifruit garden were studied [41]. Seventeen adult female participants (average age: 46.1 years) went to a kiwifruit garden, sat on a chair, and appreciated the view for 10 min. The control group viewed buildings. The results revealed that the visual stimulus of the kiwifruit garden induced higher levels of ln(HF), increased parasympathetic nervous activity, and engendered a state of physiological relaxation.

Furthermore, elderly patients requiring long-term care were selected as participants to study the effects of seated viewing of the forest, which was constructed on a hospital rooftop. This experiment showed increased parasympathetic nervous activity and decreased sympathetic nervous activity compared with the parking lot (control) on the first floor [42].

3.3. Plant Therapy

Through experience, we are all aware of the physiological relaxing effects of exposure to flowers, such as roses and foliage plants that are frequently used in flower arrangement. However, according to EBM, there is no scientific data based on physiological indicators to support the various physiological effects of plant therapy. Here we present data on the prefrontal cortex activity and autonomic nervous activity that were obtained in response to two categories of stimuli: visual and olfactory stimuli.

3.3.1. Visual Stimulation Experiment

Here, we explore the physiological relaxation effects of exposure to a visual stimulus of fresh roses. One study included a total of 114 participants, including 55 high-school students (37 males and 18 females, average age: 15.5 years), 14 medical workers (14 females, average age: 42.1 years), and 45 office workers (31 males and 14 females, average age: 38.0 years). The stimulus consisted of 30 pink, odorless roses (*Rosa*, cultivar name: Dekora), 40 cm in length. The distance between the roses and the participants was set at approximately 37–40 cm, and the duration of exposure to the visual stimulus was 4 min. The physiological indicators employed as the study parameters were HRV, measured

by fingertip accelerated plethysmography (ARTETT, U-Medica Inc., Osaka, Japan), and pulse rate, calculated by a 60/a-a interval. HF increased by 16.7% and the LF/HF ratio decreased by 30.5% in the 55 high school students in response to the visual stimulus of fresh flowers. HF also increased by 33.1% in the 14 medical workers and by 21.4% in the male office worker group [43]. The results on the total group of 114 participants showed an average increase of 15.1% in HF and a decrease of 16.3% in the LF/HF ratio. From this, we can conclude that visual stimulation with odorless fresh roses (1) increases

activity to alleviate stress state. Furthermore, the relaxation effects of foliage plants commonly found in homes and offices were studied in a total of 85 high-school students (41 males and 44 females, average age: 16.5 years) [44]. The houseplants used as the visual stimuli consisted of three pots of the striped dracaena (*Dracaena deremensis*), which stood at a height of approximately 55–60 cm from the bottom of the pot. The participants were exposed to the visual stimulus of the plants for 3 min at a distance of approximately 55 cm from the plants. The results showed that HF of the participants increased by 13.5% compared with that of the controls not exposed to the plants. The LF/(LF + HF) ratio, which reflects sympathetic nervous activity, decreased by 5.6% compared with that of the controls. Again, visual stimulation with dracaena, a common foliage plant, was proven to have physiological relaxation effects.

parasympathetic nervous activity to render a state of relaxation and (2) decreases sympathetic nervous

Next, an experiment was conducted to compare the effects of fresh and artificial pansies [45]. The participants were 40 high-school students (19 males and 21 females, average age: 16.4 years). Fresh and artificial yellow pansies grown or mounted in a planter were employed as the visual stimuli for 3 min. Compared with artificial pansies, visual stimulation with fresh pansies resulted in a decrease in the LF/HF ratio, thus demonstrating the alleviation of stress state.

Subsequently, the physiological relaxation effects of three-dimensional (3D) images of nature were studied [46]. Visual stimulation by exposure to images depicting nature is a convenient means to get in contact with nature in our modern, stress-laden society. As such, realistic 3D images are becoming popular. Visual stimulation with 3D images of natural sceneries was expected to have a different effect on the body compared with stimulation by two-dimensional (2D) images. Images of water lilies in 2D and 3D were shown for 90 s each to 19 male participants (average age: 22.2 years). The visual stimulus measured 1.7 \times 3.0 (H \times W) m, and the distance between the display and the participants was set at 2.5 m. Autonomic nervous activity by HRV and prefrontal cortex activity by near-infrared spectroscopy were measured as indicators of the physiological response. In HRV, the R-R interval was measured on a portable electrocardiographic monitor, and the maximum entropy method (MemCalc/Win, GMS, Tokyo, Japan) was used for analysis. The right and left prefrontal cortices were measured for oxygenated hemoglobin levels using near-infrared spectroscopy (NIRO-200, Hamamatsu Photonics K.K., Hamamatsu, Japan). The results showed that exposure to realistic 3D images of nature decreased ln(LF/HF) and decreased oxygenated hemoglobin levels in the right prefrontal cortex compared with 2D images, thereby showing the physiological relaxation effects of this form of natural visual stimulation.

We also compared the activation of the prefrontal cortex during presentation of real foliage plants with a projected image of the same foliage plants [47]. While viewing the plants and images, oxy-hemoglobin (oxy-Hb) concentrations in the prefrontal cortex were measured using near-infrared time-resolved spectroscopy measured by the TRS-20 system (Hamamatsu Photonics K.K., Hamamatsu, Japan). Compared with a projected image of foliage plants, viewing the actual foliage plants increased oxy-hemoglobin concentrations in the prefrontal cortex. Humans responded differently to the presentation of actual plants compared with images of these plants.

3.3.2. Olfactory Stimulation Experiment

The physiological effects of olfactory stimulation with rose and orange essential oils were studied [48] in 20 female university students (average age: 22.5 years). The stimulus was the scent of

the essential oils made from roses and orange zest. The stimulation was counterbalanced with no odor (air) as the control. The concentration of the essential oils was adjusted to vary from "slight smell" to "weak smell", and the duration of the stimulation was 90 s. The physiological indicator was the level of oxygenated hemoglobin in the prefrontal cortex, measured using near-infrared time-resolved spectroscopy (Hamamatsu Photonics K.K., TRS-20, Hamamatsu, Japan). The results showed that olfactory stimulation by essential oils from roses and orange zest decreased oxygenated hemoglobin levels in the right prefrontal cortex.

Next, the relaxation effects of olfactory stimulation with fresh roses were studied [49]. The strength of perceptibility of the stimulus was determined as "weak" or "easily" sensed and the duration of stimulation was 90 s. Increased HF and a trend toward a decrease in LF/HF were observed over the 90 s interval as a result of olfactory stimulation with fresh roses.

Furthermore, the effects of olfactory stimulation with essential oils of perilla were also studied [50]. Perilla is an herb that has been used as an accompaniment to sashimi and other dishes in Asia and has also been widely employed for its medicinal value. It is a common ingredient in antidepressant medications in traditional Chinese medicine and is thus used as a medicinal herb in modern times as well. However, much of its physiological relaxation effects are yet to be defined. To research the effects of this olfactory stimulus, 19 female university students (average age: 21.6 years) were exposed to perilla essential oils, whereas the control group was not exposed to any odors (air). The concentration of the essential oil was determined as "weak" or "easily" sensed and the duration of the stimulation was 90 s. The physiological indicator was the concentration of oxygenated hemoglobin in the prefrontal cortex, measured using near-infrared time-resolved spectroscopy (Hamamatsu Photonics K.K., TRS-20, Hamamatsu, Japan). The results showed that oxygenated hemoglobin levels in both the right and left prefrontal cortices decreased in the last third of the 90 s period in the participants compared with the control group. Thus, these results demonstrate the calming effects of olfactory stimulation with perilla essential oils on prefrontal cortex activity.

3.4. Wooden Material Therapy

Wood is a natural material that has been deeply rooted in Japanese sensibilities. Empirically, it has been perceived as a material with relaxing properties. However, the accumulation of data on the activities of the brain, autonomic nervous system, and endocrine and immune systems supported by EBM principles is extremely limited.

Here, physiological responses to stimulation with wood will be shown by presenting experiments that were primarily conducted indoors.

3.4.1. Visual Stimulation Experiment

Previous studies have reported on the physiological changes caused by visual stimulation with wooden materials in indoor rooms and its effect on continuous blood pressure and pulse rate as physiological indicators [51–53]. An actual room (13 m²) was built for the study, and the effects of visual stimulation were measured over a 90 s stimulation period as the percentage of wood in the structure and design of the room changed. Common wooden living rooms in Japan contain approximately 30% wood in their structure. A common wooden living room (30% wood) and a living room with extra wood added to the walls (45% wood) were compared. Reductions in pulse rate and diastolic blood pressure were observed, indicating that common wooden living rooms containing 30% wood have visually calming physiological effects. An increase in pulse rate was observed in living rooms containing 45% wood. In a 30% wooden room with the addition of wooden pillars and crossbeams, the pulse rate increased in a manner similar to that in the room with 45% wood, thereby showing a state of physiological wakefulness. From the above, it was clarified that the physiological changes were induced by variation in the wood percentage or design in a wooden living room.

Sakuragawa et al. [54] have demonstrated the different physiological responses to visual stimulation of full-sized Hinoki wall panels and a white steel wall panel (control). Fourteen male

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college students viewed both wall panels for 90 s. Participants were also asked to rate the wall panels according to whether they liked them or not. During the visual stimulation from the Hinoki wall panels, systolic blood pressure decreased in the participant group that evaluated the Hinoki wall panels as "like", and there was no change in the participant group that evaluated them as "dislike". For the white steel wall panels, systolic blood pressure increased in the participant group that evaluated them as "dislike".

3.4.2. Olfactory Stimulation Experiment

Japanese cedar and Japanese cypress wood chips, essential oil, and essential oil constituents were used as substances for olfactory stimulation. The odors were administered by means of an essential oil inhalation device. The experiment was conducted on participants seated in an indoor artificial climate chamber with the temperature, humidity, and illuminance set at 25 °C, 60%, and 50 lx, respectively. The strength of perceptibility of the stimulus was adjusted to a "weak smell", and the duration of the stimulation was approximately 60–90 s. The results showed a decreased systolic blood pressure and a calming effect on the prefrontal cortex activity in response to olfactory stimulation with cedar materials and cypress chips; therefore, this olfactory stimulation had an overall physiologically relaxing effect [55].

It is reported that volatile organic compounds (VOCs) emitted from Japanese cedar (Cryptomeria japonica) interior walls induce physiological relaxation [56]. Sixteen male university students (average age: 23.5 years) performed arithmetic tasks both in the absence (control) and the presence of VOCs emitted from the Japanese cedar for repeated cycles of 15 min of work and 5 min of rest. Salivary chromogranin A (CgA) concentration at the post-work measurement was higher compared with the pre-work measurement under the control condition. However, the change under the Japanese cedar condition between pre- and post-work measurements was not significant. The authors are suggesting that VOCs emitted from Japanese cedar suppresses activation of an increase in salivary CgA secretion. Furthermore, the effects of the essential oil of the Pinaceae (Abies sibirica) during and after performance of a sustained task on a visual display terminal (VDT) have been examined [57]. Nine male university students (average age: 22 years) performed the VDT work for 30 min twice, at first in the absence (control) and next in the presence of volatiles from the essential oil of Pinaceae. After VDT work, the mean R-R intervals were higher in the Pinaceae condition than the control. This meant that the heart rate was slower. There were significant differences in electroencephalogram (EEG) results as well. The EEGs recorded from the C3 and C4 electrode sites of the international 10-20 system were analyzed. After VDT work, the theta band powers were increased and the alpha band powers were decreased in the Pinaceae condition compared with the control. The arousal level from VDT work was decreased by the essential oil of Pinaceae.

On the other hand, differential results have also been reported. The influence of olfactory stimulation of Hiba (*Thujopsis dolabrata*) odor on contingent negative variation (CNV) was investigated [58]. Five milliliters of undiluted Hiba oil were warmed in an oil warmer with a 15 W incandescent lamp. In the control condition, only the lamp was on. Sixteen females were exposed to the Hiba oil and control conditions for over 30 min. Compared to the control, the amplitude of the CNV components were larger and reaction time to the imperative stimulus was shorter in the Hiba oil condition. Although the sensory intensity was not indicated, there is a possibility that the inhalation concentration of the Hiba oil was high.

Single substance inhalation experiments of α -pinene and limonene, which are the main volatile components of most wood materials and forests, have also been conducted. The blood pressure of the participants was measured every second throughout the 90 s duration of stimulation. The results showed that inhalation of α -pinene and limonene decreased systolic blood pressure [55]. Furthermore, increased parasympathetic nervous activity and decreased heart rate were also reported on olfactory stimulation with limonene [59].

As a natural construction material, wood normally needs to be thoroughly dried before use in order to prevent warping, and this is most often done by artificial drying methods. However, these processes degrade the "natural scent of wood" by altering the natural properties of the wood constituents by exposure to high temperatures or by loss of the low boiling point components. Therefore, the effects of inhaling odorous substances from chips made of "air-dried cypress wood" (produced through natural drying processes to preserve the "scent of wood") and "high-temperature-dried cypress wood" (exposed to high-temperature, high-speed drying) were compared and shed light on the differences in the effects on the left and right prefrontal cortex activity [60]. The experiment was conducted with 19 female university students (average age: 22.5 years) in an artificial climatic chamber. The strengths of perceptibility of the stimuli were adjusted to vary from "slight smell" to "weak smell". The results demonstrated that olfactory stimulation with "air-dried cypress wood" had a greater calming effect on the left and right prefrontal cortices of participants compared with the inhalation of odorous substances originating from "high-temperature-dried cypress wood".

3.4.3. Tactile Stimulation Experiment

A study on the effects of contact with wood or artificial substances on the systolic blood pressure [61] has previously been conducted. It was shown that contact with a metal board caused great fluctuations in the systolic blood pressure, whereas contact with cypress and cedar wood caused little fluctuation.

Sakuragawa et al. [62] have demonstrated the following: (1) contact with a metal board increased blood pressure, but the increase was inhibited when the metal was warmed; (2) contact with an acrylic board increased blood pressure and the rate of increase in blood pressure was greater when the acrylic board was chilled; and (3) blood pressure did not change in response to contact with cedar, cypress, or oak materials. Furthermore, it did not increase even if the oak material was chilled. These results showed that temperature conditions have a sizable effect on blood pressure increases if contact is made with artificial materials, such as metals and acrylic, whereas contact with wood does not cause physiological stress, which manifests itself in the form of blood pressure increase, regardless of its temperature. This was true whether tested at room or lower temperature, thereby showing the superiority of nature-derived wood as a material.

4. Individual Differences in the Physiological Relaxation Effects of Nature Therapy

It is known that great individual differences are observed in physiological data collected in research on stress and relaxation, but few methods have been proposed to elucidate this variability.

We used two methods to elucidate the variability phenomenon: (1) "law of initial value" and (2) "personalities", such as "Type-A behavioral patterns" and "trait anxiety". We introduce the approach method and analyzed data below.

4.1. Approach of the "Law of Initial Value"

The "law of initial value" was advocated by Wilder [63,64], and it describes the principle that the direction of the response to a stimulus depends largely on the initial value. Therefore, the higher the initial value, the smaller the response to function-raising stimuli and the larger the response to function-depressing stimuli. Lacey [65] examined the correlation between initial values and changes in systolic/diastolic blood pressure and heart rate caused by a stressor and reported that participants whose initial values were high responded poorly to function-raising stimuli, such as a stressor. Hord et al. [66] demonstrated the relationship between initial values and changes in heart rate caused by a stressor. They found that there were significant correlations between initial values and changes in heart rate and respiratory rate. Previous studies have been conducted mainly with regard to the relationship to the response by function-raising stimuli and the

initial value [65–69]. Studies to clarify the relationship between the initial value and the response by function-depressing stimuli, such as natural therapy, hardly exist.

This research investigated the individual differences in physiological relaxation effects from forest therapy from the perspective of "law of initial value" with the purpose of clarifying the physiological adjustment effects of forest therapy [70]. The experiment was conducted between 2012 and 2013 in forest and urban areas in eight locations across Japan. The indicators measured were blood pressure and pulse rate. The sample in each experiment location included 12 male university students in their 20 s, and a total of 92 participants for whom data could be obtained (average age: 21.5 years). They were randomly assigned to two groups of six persons each. One group spent Day 1 in the forest and the other in the urban area; on Day 2, the two groups switched the experiment locations. Commercial areas around Japan Railway stations were selected as the urban areas, and participants took 15 min walks in the forest and urban areas.

More participants presented with blood pressure and pulse rate reductions from walking in the forest; however, these parameters also increased in some participants, thereby showing that there is great individual variation (Figure 2a). First, the diastolic blood pressure was studied on the basis of the "law of initial value". Figure 2b shows the relationship between "initial value (absolute value before walking in the forest)" and "the change (value after walking in the forest)–(value before walking in the forest)". As shown in this figure, there is a negative correlation between the "initial value" and the "change", showing that values decreased by walking in the forest in participants whose initial values were high, and values increased in participants with low initial values. Meanwhile, there was no correlation between the "initial value" and the "change" in Figure 3, which shows the results of the same participants in the urban area. The results were similar in walking the relationship between the "initial value" and the "change" for pulse rate. In other words, it was concluded that walking in the forest has physiological adjustment effects that bring the diastolic blood pressure and pulse rate closer to the ideal values.

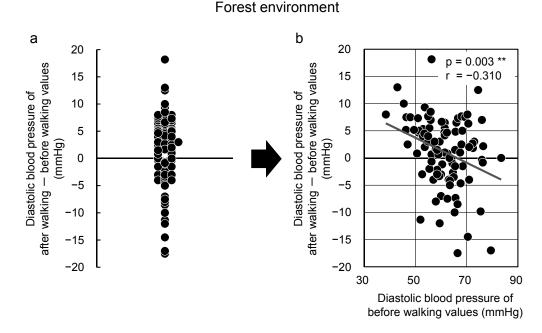
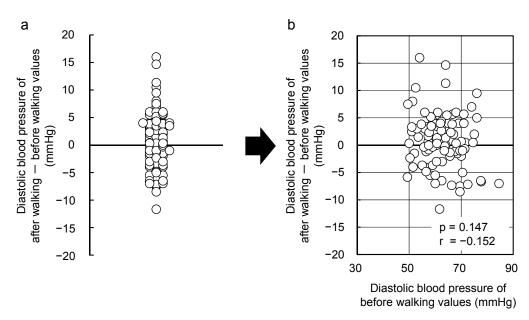


Figure 2. Changes observed with respect to walking in a forested area. Individual differences (**a**) and the relationship between the "initial value" and the "changes after walking in a forested area" (**b**) with respect to diastolic blood pressure (n = 92). ** p < 0.01 by Pearson correlation test [70].



Urban environment

Figure 3. Changes observed with respect to walking in an urban area. Individual differences (**a**) and the relationships between the "initial value" and the "changes after walking in an urban area" (**b**) with respect to diastolic blood pressure (n = 92). Pearson correlation test [70].

Conversely, in terms of previous studies on the "law of initial value", Tsunetsugu and Miyazaki [71] measured salivary cortisol levels before and after a 15 min walk in the forest and reported a negative correlation between the initial value and the change. Lee et al. [13] also showed a negative correlation between the values before and after a 15 min walk in the forest and changes in salivary immunoglobulin A (IgA) levels. IgA levels decreased in the group with a higher initial value, and there was a negative correlation between the initial value and the change after forest therapy.

4.2. Approach Employing "Personality"

Past studies on individual variations have approached the topic through a behavioral pattern category called the "Type-A behavior pattern" [72–74]. The Type-A behavior pattern can be defined as an overt behavioral syndrome related to a lifestyle that is characterized by excessive competitiveness, striving for achievement, aggressiveness, time urgency, acceleration of common activities, restlessness, hostility, hyperalertness, explosiveness of speech amplitude, facial musculature tension, feelings of struggling against the limitations of time, and insensitivity to the environment [75,76].

Here, "personality" was used to organize differences in individual physiological responses to forest therapy. In seated forest-viewing experiments conducted in 44 locations nationwide, 485 participants were divided into Type-A and Type-B groups. The differences in their responses were studied [73]. The results showed that 15 min of seated viewing of the forest decreased the pulse rate by 2.2 beats/min in the 485 participants. Compared with the reduction rate of 1.6 beats/min in the urban areas, the forest group showed a remarkable 0.6 beat/min difference in the reduction of pulse rate. Next, the participants were categorized into two groups: Type-A and Type-B behavior patterns (233 and 252 participants, respectively). The pulse rate of the Type-B group decreased from 65.7 beats/min to 63.2 beats/min, showing a 2.5 beats/min decrease from seated viewing of the forest, whereas no significant change was observed in the Type-A group. Finally, the Type-A and Type-B groups were divided again to form four subgroups. The low-score Type-B group showed a decrease of 1.7 beats/min in the forest compared with that in the urban area; however, in the other three groups, there was no significant change between the forest and urban area groups. In conclusion, in comparison with the urban area, the change in the pulse rate from 15 min of seated viewing in the forest (1) greatly decreased

the heart rate in all 485 participants; (2) it largely decreased the heart rate in Type-B participants, but no difference was observed in the Type-A participants; and (3) after dividing the whole group into four subgroups, a large reduction was observed only in the low-score Type-B group. On the basis of these results, the study demonstrated that forest therapy can decrease the pulse rate but this reduction depends largely on the participant's personality; furthermore, individual variability related to changes in pulse rate in the forest therapy may be explained by personality categories.

5. Conclusions

This review presented scientific data to elucidate the physiological relaxation effects of nature therapy on activities of the central nervous system, autonomic nervous system, endocrine system, and immune system on the basis of advances in various physiological indicators from the viewpoint of EBM in Japan. Furthermore, our methods to approaching individual differences that arise in these measures were also described.

In Japan, the therapeutic effects of the stimulation of nature have always been known empirically, but because of the lack of data, submission of scientific data was socially demanded, and since then, there has been a gradual progression. The present review showed the current state of data accumulation. However, several limitations exist. First, most of the studies use short time periods of stimulation. In the future, long-term data over days, weeks, and months are needed. Second, there are many studies on men and women in their 20s. To generalize the findings, further studies based on a larger sample, including various age groups, are required. In addition, studies in people with premorbid conditions are also required. Finally, it is necessary to comprehensively evaluate the parameters that are used as indicators of brain activity, autonomic nervous activity, endocrine activity, and immunization activity.

Considering the significance of quality of life in our modern stressful society, the importance of nature therapy will further increase. The therapeutic effects of natural stimulation suggest a simple, accessible, and cost-effective method to improve the quality of life and health of modern people. Moreover, the elucidation of these physiological effects from the viewpoint of EBM is an important task for the future.

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Abbreviations

The following abbreviations are used in this manuscript:

2D	Two-dimensional
3D	Three-dimensional
EBM	Evidence-based medicine
EEG	Electroencephalogram
HRV	Heart rate variability
IgA	Immunoglobulin A
NK	Natural killer
LF	Low frequency
HF	High frequency
SD	Semantic differential
TRS	Near-infrared time-resolved spectroscopy
VOCs	Volatile organic compounds
VDT	Visual display terminal

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Article Effects of Visual Stimulation with Bonsai Trees on Adult Male Patients with Spinal Cord Injury

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Abstract: Nature therapy has been demonstrated to induce physiological relaxation. The psychophysiological effects of nature therapy (stimulation with bonsai trees) on adult male patients with spinal cord injury (SCI) were examined. Oxyhemoglobin concentration changes in the prefrontal cortex were measured using near-infrared spectroscopy, and heart rate variability was analyzed. Psychological responses were evaluated using the modified semantic differential method and Profile of Mood States (POMS) subscale scores. Visual stimulation of adult male patients with SCI elicited significantly decreased left prefrontal cortex activity, increased parasympathetic nervous activity, decreased sympathetic nervous activity, increased positive feelings, and resulted in lower negative POMS subscale scores. Nature therapy can lead to a state of physiological and psychological relaxation in patients with SCI.

Keywords: spinal cord injury; nature therapy; bonsai trees; visual stimulation; near-infrared spectroscopy; heart rate variability

1. Introduction

Stress appears to be increasingly present in our modern and demanding industrialized society. Every aspect of our bodies and brains can be virtually influenced by stress induced by living in an urban environment [1]. Early human civilizations lived in natural settings, demonstrating that we can adapt to nature. Congruent with this viewpoint, individuals living in modern societies who are experiencing stress have become interested in several types of natural therapy [2]. For example, psychological evaluations of the effects of horticultural therapy on the elderly have been previously reported [3,4].

The progress and development of research involving nature and forest medicine has advanced in recent years with the development of medical equipment related to the natural and life sciences. For example, oxyhemoglobin (oxy-Hb) concentrations in the prefrontal cortex were measured using a portable near-infrared spectroscopy (NIRS) device, which revealed that foliage plants can have physiological relaxation effects in male participants [5]. Heart rate variability (HRV) was measured, which revealed that visual stimulation with roses increased parasympathetic nervous activity [6] and that fresh pansies decreased sympathetic nervous activity [7]. Furthermore, salivary cortisol levels were measured after the participants were subjected to gardening activity, which demonstrated decreased stress levels [8]. A review by Song et al. presented scientific data to elucidate the physiological relaxation effects of nature therapy on the activities of the central nervous system, autonomic nervous system, endocrine system, and immune system [9]. The results from these experiments are based on advances in various physiological indicators from the viewpoint of evidence-based medicine in Japan. Nature therapy has the potential to be more widely adopted as preventive medicine in the future.

One potential application of nature therapy is its use for patients with spinal cord injury (SCI). SCI is a devastating event for individuals, and they frequently develop motor and sensory impairments, as well as autonomic dysfunction. Previous studies have reported that autonomic nervous activity plays a major role in social cognition and that difficulties in the ability to interpret social information are commonly observed in a variety of mental disorders, which in turn correlate with poor autonomic nervous system regulation [10]. Depressive disorders are the most frequent concern following SCI and significantly affect rehabilitation, community integration, quality of life (QOL), and health-related outcomes [11–13]. A clinical practice guideline published in 1998 noted that 25% of men and 47% of women with SCI experienced some form of depressive disorder [14].

Considering this high prevalence of psychological distress, it is especially important to highlight that according to research, most patients with SCI felt that their emotional needs were not sufficiently addressed by their rehabilitation team [15]. Recent meta-analyses have reported medium-to-large effect sizes for psychological interventions for post-SCI depression, and there is sufficient evidence specifically supporting the use of cognitive behavioral therapy interventions [16]. However, these methods require the intervention of an expert psychiatrist.

An advantage of nature therapy such as viewing bonsai trees is that it allows for routine, self-induced mental relaxation. Such therapy is also accessible for individuals who are unable to perform certain activities (e.g., walking more than a mile or doing vigorous activities). If the physical and mental stress of patients with SCI can be reduced via intervention with nature therapy, this therapy can be recommended to such patients to promote improved health. Similarly, nature therapy can be used as a preventive medicine therapy for healthy but stressed individuals. Relaxing effects have been reported regarding exposure to forest, urban green space, flowers and plants, and so on. Nature therapy is defined as "a set of practices aimed at achieving 'preventive medical effects' through exposure to natural stimuli that render a state of physiological relaxation and boost the weakened immune functions to prevent disease" [9].

Although previous analytical studies have pointed out the relevance of nature therapy and relaxation in healthy adults, there is no previous research on adult patients with SCI. To the best of our knowledge, this is the first study to examine the physiological and psychological effects of nature therapy in adult male patients with SCI and clarify its effectiveness in reducing daily stress.

In this study, 24 Japanese adult male patients with chronic-stage SCI were exposed to 10-year-old cypress bonsai trees as visual stimuli. Bonsai is miniature natural landscapes in pots using trees and other plants. They are a famous art form unique to Japan.

2. Materials and Methods

2.1. Experimental Design

All participants gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Center for Environment, Health and Field Sciences, Chiba University, Japan (Project identification code number: 5). In total, 24 Japanese male patients with spinal cord injury aged 25–79 years (mean age, 49.0 ± 16.4 years) were included in this study. They had a height of 162–182 cm (171.4 ± 5.6 cm) and weight of 52–94 kg (67.2 ± 9.2 kg). The patients had no psychiatric disorders, which comprised part of the inclusion criteria for the study, and they were in the chronic stage of their condition (i.e., >1 year after the lesion developed). They were diagnosed with spinal cord injury by a doctor and their damage was located below C7. The patients were able to independently move around in wheelchairs.

The experiments were conducted in an experimental room at Chiba University. The room temperature was maintained at 23.7 ± 1.3 °C, and the relative humidity was maintained at $50.5 \pm 7.4\%$. The patients moved into the experimental room and the experiments were separately carried out for each patient.

Miniature potted 10-year-old Japanese cypress bonsai trees were used as visual stimuli. Eight cypress trees, approximately 55 cm in height, were grouped together in a $40 \times 20 \times 5$ cm ceramic pot (Figure 1A). Before visual stimulation, these miniature trees were covered by a corrugated cardboard box (rest condition). After a 60-s rest period, the patients viewed the miniature potted trees (visual stimulation) or nothing (control) for 60 s each; all patients were made to experience both experimental conditions. Distance from the patients' eyes to the trees was 60–63 cm. The order of conditions (i.e., visual stimulation vs. control) was randomized. The patients practiced the procedure, using visual stimulation with a potted plant, once beforehand.

2.2. Physiological Indices

Changes in oxy-Hb concentrations on the surface of the prefrontal cortex were measured using a two-channel near-infrared spectroscopy device (NIRS; Pocket NIRS Duo, DynaSense, Hamamatsu, Japan). NIRS probes were placed bilaterally and symmetrically on the forehead. Two sensors were placed over the frontal region, with one sensor placed on the left side of the forehead and the other placed on the right side of the forehead (Figure 1B) [17]. To analyze the NIRS response, change in the oxy-Hb concentrations in the prefrontal cortex during visual stimulation was measured. The difference between this and the value from 10 s prior to stimulation was analyzed. It is well established that oxy-Hb concentration reflects the activation of neural regions [18].

The patients placed their left forefingers on the sensor of an accelerated plethysmograph (ARTETT, U-Medica Inc., Osaka, Japan) (Figure 1C). Heart rate variability (HRV) was analyzed. HRV was converted by a 60/a-a interval; the sampling frequency was 1000 Hz. The power levels of the high-frequency (HF) (0.15–0.40 Hz) and low-frequency (LF) (0.04–0.15 Hz) components were calculated using the maximum entropy method [19,20]. HF power was considered to reflect parasympathetic nervous activity, and the LF to HF ratio was considered to reflect sympathetic nervous activity [21,22]. In general, parasympathetic nervous activity is enhanced during relaxation and sympathetic nervous activity is enhanced at the time of awakening or in situations of stress.

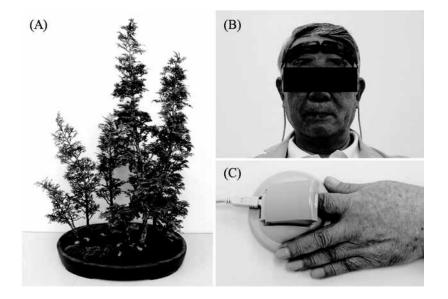


Figure 1. Bonsai trees and physiological measurement apparatuses. (**A**) Japanese cypress bonsai trees; (**B**) Participant undergoing near-infrared spectroscopy (NIRS) measurement; (**C**) Participant undergoing heart rate variability (HRV) measurement.

2.3. Psychological Indices

The modified semantic differential (SD) method and Profile of Mood States (POMS) subscale scores were used to evaluate psychological responses following visual stimulation. The modified SD method uses three pairs of adjectives anchoring 13-point scales: "comfortable to uncomfortable," "relaxed to awakening," and "natural to artificial" [23]. The scores were determined for the following six POMS subscales: "tension-anxiety (T-A)," "depression (D)," "anger-hostility (A-H)," "vigor (V)," "fatigue (F)," and "confusion (C)." A short form of POMS with 30 questions was used to decrease participant burden [24–26]. The "total mood disturbance (TMD)" score was calculated by [(T-A + D + A-H + F + C) – V]. A high TMD score indicates an unfavorable psychological state.

2.4. Statistical Analysis

We used paired *t*-tests to compare physiological indices and the Wilcoxon signed-rank test to compare psychological test scores. All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Data are expressed as means \pm standard error (mean \pm SE). For all cases, *p* < 0.05 (one-sided) was considered statistically significant. One-sided tests were used because we hypothesized that the patients would be relaxed after viewing the bonsai trees.

3. Results

Oxy-Hb concentrations of the left and right prefrontal cortices were measured using a two-channel NIRS device. Change in the oxy-Hb concentration of the left prefrontal cortex was significantly lower when the patients viewed the bonsai trees (visual stimulation) than when they viewed nothing (control) (visual stimulation = $-0.20 \pm 0.02 \mu$ M; control = $0.17 \pm 0.02 \mu$ M; *p* < 0.05; Figure 2A). Oxy-Hb concentration of the right prefrontal cortex did not significantly differ between visual stimulation ($0.00 \pm 0.01 \mu$ M) and control ($0.09 \pm 0.01 \mu$ M; Figure 2B) conditions.

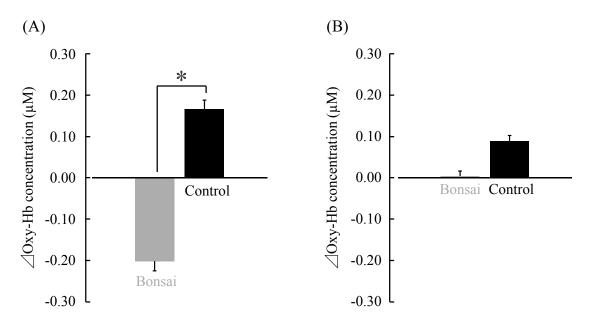


Figure 2. Mean oxy-Hb concentrations in the prefrontal cortices. (**A**) Changes in the left prefrontal cortex when viewing bonsai trees vs. control; (**B**) Changes in the right prefrontal cortex. N = 24, mean \pm standard error. * p < 0.05, paired *t*-test. Δ , change.

The average power of the high-frequency (HF) components of HRV, which is related to parasympathetic nervous activity, increases when we feel relaxed [21,22]. This value was significantly greater when the patients viewed the bonsai trees compared with the control condition (visual stimulation = 5.45 ± 0.23 lnms²; control = 4.95 ± 0.21 lnms²; p < 0.01; Figure 3A). The average

low-frequency (LF) to HF ratio of HRV, which is related to sympathetic nervous activity, increases when we feel stressed [21,22]. This ratio was significantly lower when the patients viewed the bonsai trees compared with the control condition (visual stimulation = 0.85 ± 0.04 ; control = 0.95 ± 0.06 ; p < 0.01; Figure 3B).

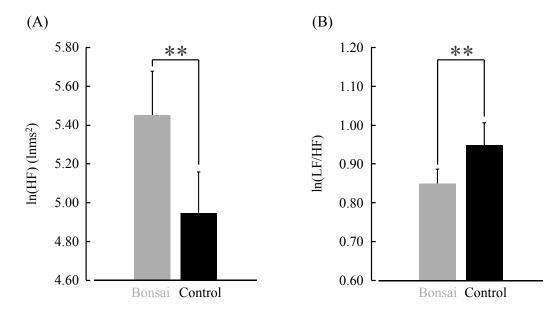


Figure 3. Autonomic nervous activity when viewing bonsai trees vs. control. (**A**) Parasympathetic nervous activity: mean natural logarithm (ln) of the high-frequency (HF) component; (**B**) Sympathetic nervous activity: mean natural logarithm (ln) of the ratio of low-frequency (LF) to HF (LF/HF). N = 24, mean \pm standard error. ** p < 0.01, paired *t*-test.

Figure 4A shows the results of the modified SD (semantic differential) method. Subjective evaluations indicated that the patients felt significantly more "comfortable," "relaxed," and "natural" when viewing the bonsai trees compared with the control condition (p < 0.01). The Profile of Mood States (POMS) was used to gauge the patient's psychological response to stimuli (Figure 4B). Negative POMS subscale scores of "tension-anxiety," "depression," "confusion," "anger-hostility," and "fatigue" were significantly lower when viewing the bonsai trees compared with the control condition (p < 0.05). On the other hand, the scores of "vigor," a positive subscale, were significantly higher when viewing the bonsai trees compared with the control condition (p < 0.01). The scores of global "total mood disturbance" were significantly lower when viewing the bonsai trees compared with the control condition (p < 0.01); indeed, negative emotions were significantly reduced when the patients were exposed to natural stimuli.



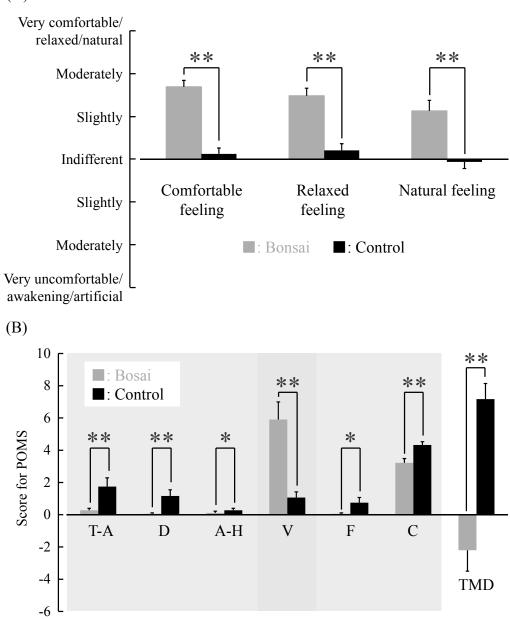


Figure 4. Questionnaire results. **(A)** Subjective feelings measured using the modified semantic differential method after viewing the bonsai trees vs. control. N = 24, mean \pm standard error. ** p < 0.01, Wilcoxon signed-rank test; **(B)** Scores on the Profile of Mood States after viewing the bonsai trees vs. control. T-A, tension-anxiety; D, depression; A-H, anger-hostility; V, vigor; F, fatigue; C, confusion; and TMD, total mood disturbance. N = 19, mean \pm standard error. * p < 0.05 and ** p < 0.01, Wilcoxon signed-rank test.

4. Discussion

Research demonstrates that oxygen consumption, regional cerebral blood response, and oxy-Hb supply are increased in highly activated neural regions [18]. A lower oxy-Hb concentration indicates that the quantity of oxygen transmitted to the prefrontal cortex tissue is small. The lower prefrontal cortex activity found in the current study is consistent with that reported in previous studies [27,28], showing that low oxy-Hb concentrations represent the calming of brain activity. For example, in the dorsolateral prefrontal cortex, hemispheric specialization of emotional processing has been proposed

by functional magnetic resonance imagining. In particular, the activation of the left prefrontal cortex has been associated with positive mood and the processing of positive stimuli, whereas the activation of the right prefrontal cortex has been linked to negative mood and the processing of negative stimuli [29]. However, the precise details of the function of the right and left prefrontal cortex as measured using the NIRS device remain unknown. In this experiment, the activity of the left prefrontal cortex was suppressed, whereas that of the right prefrontal cortex did not change when the patients viewed the bonsai trees. Based on these results, we can only conclude that the patients were in a relaxed state when they viewed the bonsai trees.

Patients with SCI have reduced autonomic flexibility, as measured using HRV, and exhibit reduced autonomic modulation during emotion recognition tasks [10]. However, in the current study, the patients with SCI showed significantly higher parasympathetic nervous activity and significantly lower sympathetic nervous activity when exposed to a natural stimulus. These results demonstrate that in patients with SCI, the autonomic nervous system responds to natural stimuli in a similar manner to that in healthy adults [9,30].

Patients with SCI report pain-related disability, depression, fatigue, pressure sores, spasticity, and issues with bladder and bowel management [31]. These conditions often induce negative mood states in patients with SCI; thus, emotional support is an important factor influencing the rehabilitation process [32]. The focus of rehabilitation for such patients has shifted from medical management to QOL issues, and exposure to natural stimuli represents one way to improve QOL in patients with SCI.

Viewing bonsai trees simulates "forest therapy," a therapeutic activity that has become popular in Japan, and utilizes the scientifically proven effects of walking through and viewing forests [33,34]. Indeed, forest therapy is increasingly recognized as a relaxation and stress management strategy with demonstrated clinical efficacy. Forest therapy suppresses sympathetic nervous activity, increases parasympathetic nervous activity, and reduces cortisol levels and cerebral blood flow in the prefrontal cortex [9]. Forest therapy has also been shown to increase human natural killer cell activity and improve immunity [35–37], and these effects have been proven to last for at least seven days [36,37]. In addition, psychological studies have demonstrated the benefits of forest environments on subjective measures of stress, cognitive function, and mood [38]. There is a difference between forest therapy and this experiment; namely, one is performed in the field and the other indoors. The field experiment reveals the general influence of nature on humans through their five senses. On the other hand, we can pick up the effect of stimulating a single sense in the indoor experiment and clarify its influence. Here, we chose vision as one of the senses that we use in the forest, and we clarified the influence of visual stimulation by bonsai trees.

In the current study, the same psychophysiological effects of visual stimulation with bonsai trees were shown in adult male patients with SCI as in healthy adults. We consider it an important point that a relaxation effect could also be obtained in SCI patients by applying nature therapy, because it is difficult to perform forest therapy with SCI patients with restricted activities. Further, these findings may help promote the development of the environment, which is beneficial to the physical and mental health of individuals with disability.

The main limitation of the present study was its small sample size. Also, the results of the present study cannot yet be extrapolated to females. On the other hand, we compared only the condition of viewing miniature potted trees (visual stimulation) with viewing nothing (control) in this study, but it would be useful to investigate a third condition with a non-nature object. Furthermore, a short-term stimulus was used; the results were compared before and after only 60 s of stimulation with bonsai trees. In addition, we do not know for how long the effect of this natural therapy may last. Future studies examining the duration of effects following exposure to natural stimuli are required.

5. Conclusions

In conclusion, the current study revealed that visual stimulation with bonsai trees in adult male patients with SCI elicited the following: (1) significantly suppressed left prefrontal cortex activity;

(2) significantly increased parasympathetic nervous activity and decreased sympathetic nervous activity; (3) significantly increased "comfortable," "relaxed," and "natural" feelings as assessed using the modified SD method; and (4) significantly decreased negative and increased positive POMS subscale scores. The findings of this study can be applied to SCI patients by taking advantage of their natural surroundings to ensure improved health and reduced mental stress. Generally, considerably less practice is supported by research, and the reality is that little research is applied in practice. We can say that this study is useful because in practice the effects are proved by research data.

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Author Contributions: Hiroko Ochiai contributed to data acquisition, interpretation of results, and manuscript preparation. Chorong Song and Harumi Ikei contributed to the experimental design, data acquisition, statistical analysis, and interpretation of results. Michiko Imai conceived the study and participated in the interpretation of results. Yoshifumi Miyazaki conceived and designed the study and contributed to the interpretation of results and manuscript preparation. All authors have read and approved the final version submitted for publication.

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Article Physiological Effects of Viewing Bonsai in Elderly Patients Undergoing Rehabilitation

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Abstract: The benefits of various nature-derived stimuli that can be used for stress relief and relaxation has recently gained immense attention; however, there are very few studies about their influence on elderly patients. The present study aims to present the effects of viewing bonsai on autonomic nervous activity, prefrontal cortex activity, and subjective assessment findings of psychological relaxation in elderly patients undergoing rehabilitation. Fourteen participants aged 64-91 years (mean age \pm standard deviation, 78.6 \pm 9.6 years) participated in this study. Miniature potted 10-year-old Japanese cypress bonsai trees were used as visual stimuli. Participants viewed the bonsai for 1 min, and the control comprised of no experimental stimulus. Physiological effects on autonomic nervous activity were assessed by measuring the heart rate variability (HRV) and pulse rate. The effects on prefrontal cortex activity were determined using near-infrared spectroscopy, which involved assessment of oxyhemoglobin concentrations in the left and right prefrontal cortices. Subjective evaluations were achieved by the modified semantic differential method. Viewing bonsai resulted in a significant increase in parasympathetic nervous activity, a significant decrease in sympathetic nervous activity, and a significant increase in the perceptions of feeling "comfortable" and "relaxed." In conclusion, our findings indicated that viewing bonsai induces physiological and psychological relaxation.

Keywords: older adults; nature therapy; heart rate variability; near-infrared spectroscopy; semantic differential method

1. Introduction

Along with the accelerated aging of the population, interest in enhancing the quality of life of the elderly is growing as well. It is clear that further research into the reduction of morbidity is necessary and that healthy living should be increasingly emphasized [1]. The primary focus of healthcare has been shifting from disease treatment to health promotion, disease prevention, and quality of life improvement. Recent studies have reported that natural environments, such as forests and urban parks, play an important role in health promotion and that nature-derived stimuli are positively associated with human health [2–4]. "Nature therapy" is defined as "a set of practices" aimed at achieving "preventive medical effects" through exposure to natural stimuli that render a state of physiological relaxation and boosts weakened immune functions to prevent diseases [2]. This therapy is increasingly recognized as an effective relaxation and stress management approach, and has the potential to be more widely adopted as an alternative and complementary therapy in the future.

As our modern lifestyle offers limited opportunities to get exposure to the natural environment, there is growing interest in the effects of nature-derived stimuli, which can be used daily in terms of stress relief and relaxation. A simple method to achieve contact with nature in an indoor setting is exposure to plants, such as foliage plants and fresh flowers, which are commonly used to decorate homes and offices. It is known that plants not only improve the quality of indoor air [5–7], but also help facilitate physiological and psychological relaxation through visual stimulation [2,8–12]. Previous studies have shown that viewing plants can decrease oxy-hemoglobin (oxy-Hb) concentration in the prefrontal cortex [8,9]; enhance parasympathetic nervous activity, which is increased in a relaxed state; suppress sympathetic nervous activity, which is increased or stressed state; and decrease pulse rates [9–11]. Furthermore, viewing plants can increase positive feelings of comfort, relaxation, naturalness, and vigor, and decrease negative feelings of tension, anxiety, and fatigue [8–11]. A previous study examining the therapeutic effects of plants in a hospital environment found that systolic blood pressure and ratings of pain, anxiety, and fatigue were lower among patients in hospital rooms with plants than among those in rooms without plants [12].

However, the limitations that most previous studies faced were that they only investigated physiological responses associated with viewing plants in healthy young people. In a previous study, we demonstrated the physiological and psychological effects of viewing bonsai, which is a miniature natural landscape in a pot created using trees and other plants, in adult male patients with spinal cord injury [13]. We revealed that viewing bonsai significantly decreased left prefrontal cortex activity, increased parasympathetic nervous activity, decreased sympathetic nervous activity, increased positive feelings, and reduced negative feelings [13]. However, there have been no examinations on the physiological effects of viewing plants in elderly patients.

The aim of this study is to clarify the effects of viewing bonsai on autonomic nervous activity through measurements of heart rate variability (HRV) and pulse rate, prefrontal cortex activity through assessments using near-infrared spectroscopy (NIRS), and psychological relaxation through subjective assessments in elderly patients undergoing rehabilitation.

2. Materials and Methods

2.1. Participants

The study targeted Japanese elderly outpatients or hospitalized patients undergoing rehabilitation due to several conditions, such as lumbar compression fracture, femoral neck fracture, cerebral infarction, and cardiogenic cerebral embolism at Noda Hospital, Japan. The study included 14 patients (males, 4; females, 10) aged 64–91 years (mean age \pm standard deviation, 78.6 \pm 9.6 years). The height ranged from 139 to 175 cm (151.9 \pm 10.1 cm) and weight ranged from 31 to 71 kg (51.4 \pm 12.5 kg). Among the participants, patients with mild dementia were also included. All participants were informed about the aims and procedures of the study. After receiving a description of the experiment, they provided written consent to participate in the study. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Center for Environment, Health, and Field Sciences, Chiba University, Japan (project identification no.: 5).

2.2. Visual Stimulation

In this study, bonsai was used as visual stimuli. Bonsai has a characteristic of mimicking natural landscapes and is one of the nature-derived stimuli that has been used in daily life in Japan since a long time ago. In this experiment, miniature potted 10-year-old Japanese cypress bonsai trees modeling a forest landscape were used. Eight cypress trees, approximately 55 cm in height, were grouped together in a $40 \times 20 \times 5$ cm ceramic pot. The distance from the participants' eyes to the trees was 60–63 cm.

2.3. Experimental Design

Experiments were conducted in an experimental room at Noda Hospital, Japan. The room temperature, relative humidity, and illumination were 25.7 °C \pm 0.7 °C, 66.3% \pm 3.1%, and 383.0 \pm 105.7 lx, respectively. After receiving a description of the purpose and outline of the study, the participants were moved into the experimental room while being seated on a wheelchair. Sensors for physiological measurement were fitted, and the participants received a detailed description about the experimental procedure. They then practiced the procedure using a foliage plant, once before the experiment.

The study protocol is presented in Figure 1. Before visual stimulation, the bonsai was covered with a corrugated cardboard box (rest condition; Figure 1 left). After a 1 min rest period, the participants viewed the bonsai (bonsai condition; Figure 1 upper right) or nothing (control condition; Figure 1 bottom right) for 1 min. All participants experienced both experimental conditions. During the testing procedure, the participants' physiological responses were continually measured. After completion of the 1 min visual stimulation, subjective evaluations were conducted. To eliminate influences from the order of viewing the bonsai and the control, the visual stimuli were presented in a counterbalanced order.

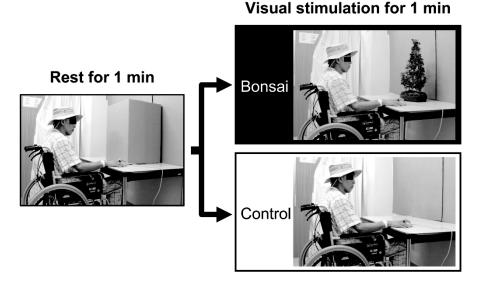


Figure 1. Experimental viewing conditions. Clockwise from left: rest condition, bonsai condition, and control condition.

2.4. Physiological Measurements

HRV and pulse rate were measured to assess autonomic nervous activity [14]. For assessment, the participants placed their forefingers on the sensor of an acceleration plethysmograph (APG; ARTETT, U-Medica Inc., Osaka, Japan). Previous studies have reported that the a–a intervals of an APG and the R–R intervals of an electrocardiograph are highly correlated [15]. Therefore, HRV was calculated by spectral analysis of the coefficient of variation of the a–a intervals of an APG. HRV was converted by a 60/a–a interval, and the sampling frequency was 1000 Hz. The power levels of the high frequency (HF; 0.15–0.40 Hz) and low frequency (LF; 0.04–0.15 Hz) components were calculated using the maximum entropy method (MemCalc/Win; GMS, Tokyo, Japan) [16]. HF power was considered to reflect parasympathetic nervous activity, and the LF-to-HF ratio (LF/HF) was considered to reflect sympathetic nervous activity [14,16]. To normalize the HRV parameters across participants, we used natural logarithmic-transformed values in the analysis [17]. In general, parasympathetic nervous activity is enhanced at the time of awakening or in situations of stress.

NIRS was used to assess brain activity [18,19]. The sensors were mounted at approximately Fp1 and Fp2 of the international 10–20 system (electroencephalogram) on each participant's forehead. The oxy-Hb concentrations in the left and right prefrontal cortices were measured using a portable NIRS device (Pocket NIRS Duo, DynaSense, Hamamatsu, Japan). It is known that an increase or decrease in the quantity of blood flow in the brain is consistent with a corresponding increase or decrease in oxy-Hb [20], and it is thought that a decrease in the oxy-Hb concentration causes physiological calming.

2.5. Psychological Measurements

For the psychological measurements, the Japanese version of the modified semantic differential (SD) method was used [21]. The SD method subjectively assesses participants through a questionnaire with opposing adjectives, each of which is evaluated on a 13-point scale. Two pairs of adjectives were assessed as "comfortable–uncomfortable" and "relaxed–awakened." Of the 14 participants, 9 had difficulties in filling out the questionnaire alone, and for these participants, hospital staff filled out the questionnaire for them after listening to their responses.

2.6. Data Analysis

All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Paired *t*-tests were used to compare physiological responses between before and after viewing bonsai (pre- vs. post-measurement) and between the two stimuli (bonsai vs. control). The Wilcoxon signed-rank test was used to compare psychological responses. Data are expressed as mean \pm standard error (mean \pm SE). For all analyses, a *p*-value < 0.05 was considered statistically significant. One-sided tests were used because we hypothesized that the participants would experience relaxation on viewing bonsai.

3. Results

Figure 2 shows the comparison of the HRV results between viewing bonsai and the control. The mean ln(HF) values are presented in Figure 2 (left). On comparing the ln(HF) values before and after viewing bonsai, we found that the ln(HF) values were significantly higher after viewing bonsai than before viewing bonsai (5.12 \pm 0.22 vs. 4.68 \pm 0.37 lnms²; *p* = 0.043). Moreover, on comparing the two stimuli, we found that the ln(HF) values were significantly higher when viewing bonsai than when viewing the control (5.12 \pm 0.22 vs. 4.51 \pm 0.33 lnms²; *p*= 0.012). The mean ln(LF/HF) values are presented in Figure 2 (right). On comparing the ln(LF/HF) values before and after viewing bonsai, we found that the ln(LF/HF) values were significantly lower after viewing bonsai than before viewing bonsai (-1.88 ± 0.23 vs. -1.19 ± 0.26 ; p = 0.004). Furthermore, on comparing the two stimuli, we found that the ln(LF/HF) values were significantly lower when viewing bonsai than when viewing the control (-1.88 ± 0.23 vs. -1.29 ± 0.26 ; p = 0.048). However, there were no significant differences in the pulse rate on comparing between before and after viewing bonsai (69.2 \pm 3.8 vs. 69.4 \pm 3.8 beats/min; p = 0.326) and between viewing bonsai and the control (69.4 ± 3.8 vs. 69.0 ± 3.6 beats/min; p = 0.274). Moreover, there was no significant difference in respiratory frequency between viewing bonsai and the control (0.29 ± 0.06 vs. 0.30 ± 0.07 Hz; p = 0.188). Furthermore, the physiological responses were not significantly different between the rest condition with the cardboard box prior to viewing the bonsai and the control.

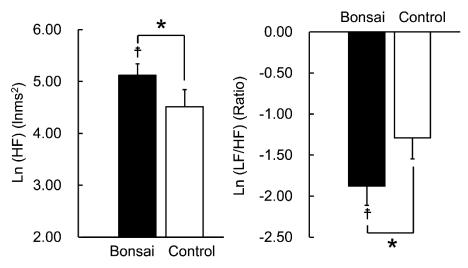


Figure 2. Heart rate variability during visual stimulation with bonsai vs. control. Left: parasympathetic nervous activity, mean natural logarithm (ln) of the high-frequency (HF) component; right: sympathetic nervous activity, mean natural logarithm (ln) of the ratio of low frequency (LF) to HF (LF/HF). Data are expressed as mean \pm standard error (n = 12). [‡] p < 0.05 (before vs. after viewing), * p < 0.05 (bonsai vs. control), according to a paired *t*-test (one sided).

There were no significant differences in the changes in oxy-Hb concentrations in the left (bonsai: $-0.14 \pm 0.01 \ \mu\text{M}$ vs. control: $-0.04 \pm 0.01 \ \mu\text{M}$; p = 0.219) and right prefrontal cortices (bonsai: $-0.02 \pm 0.01 \ \mu\text{M}$ vs. control: $-0.04 \pm 0.01 \ \mu\text{M}$; p = 0.442) between viewing the bonsai and the control.

Figure 3 shows the results of the modified SD method. Subjective evaluations indicated that the patients felt significantly more "comfortable (p = 0.004)" and "relaxed (p = 0.025)" when viewing the bonsai than when viewing the control.

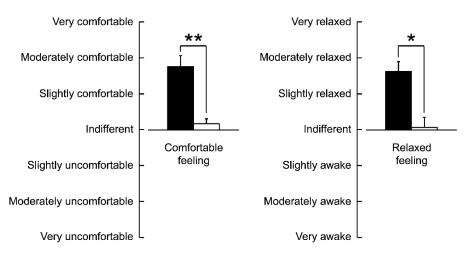


Figure 3. Subjective feelings as measured using a modified semantic differential questionnaire after visual stimulation with the bonsai vs. control. Data are expressed as mean \pm standard error (n = 14). ** p < 0.01, * p < 0.05, according to the Wilcoxon signed-rank test (one sided).

4. Discussion

This study examined the physiological effects of viewing bonsai on autonomic nervous activity assessed using HRV and pulse rate, and on left and right prefrontal cortex activities assessed using NIRS. Furthermore, subjective assessments of psychological relaxation were conducted.

Results of physiological measurements showed that viewing bonsai significantly increased parasympathetic nervous activity and decreased sympathetic nervous activity. These results are partly consistent with the findings of previous studies on the effects of viewing foliage plants, fresh

flowers, and bonsai [9–11,13]. However, there were no significant differences in pulse rate. A significant difference in HRV could be detected; however, no significant difference was detected in pulse rate because HRV is a more sensitive indicator than pulse rate. Previous studies on office workers to clarify the effects of viewing roses also showed a significant difference in HRV but not in pulse rate [11]. These results are partly consistent with the findings of the present study. Because parasympathetic nervous activity was induced, and sympathetic nervous activity was suppressed by viewing bonsai, it appears that viewing bonsai brings about physiological relaxation.

There were no significant differences in changes to oxy-Hb concentration in the left and right prefrontal cortices, and the reason for these insignificant differences in prefrontal cortex activity remains unknown. However, the oxy-Hb concentration did tend to decrease in the left prefrontal cortex when viewing the bonsai compared with the control, although the difference was not significant (bonsai: $-0.14 \pm 0.01 \mu$ M vs. control: $-0.04 \pm 0.01 \mu$ M; *p* > 0.05). It is necessary to obtain more data on the influence of plants on prefrontal cortex activity in future studies.

The results of subjective assessments of psychological relaxation showed that viewing bonsai significantly increased perceptions of feeling "comfortable" and "relaxed", compared with the absence of bonsai.

This study showed that exposure to bonsai in an indoor environment could bring about effects of physiological and psychological relaxation in elderly patients undergoing rehabilitation. As elderly patients spend little time doing outdoor activities and spend more time in indoor environments compared to healthy young people, we believe that the finding of there being positive physiological and psychological relaxation effects with observation of indoor plants through the application of nature therapy is important. Furthermore, this finding may help promote the development of the environment, which is beneficial to the physical and mental health of elderly patients.

The present study had some limitations. The main limitation was the small sample size. To generalize the findings, further studies based on a larger sample including other demographic groups are required. Furthermore, a short stimulation duration of 1 min was used, and we do not know how long the effects will last. Future studies examining the duration of the effects following exposure to natural stimuli are required.

5. Conclusions

Our findings indicated that viewing bonsai induced physiological and psychological relaxation. Thus, nature therapy should be considered in elderly patients for improving quality of life.

Author Contributions: C.S. contributed to the experimental design, data acquisition, statistical analysis, interpretation of results, and manuscript preparation. H.I. contributed to the experimental design, data acquisition, statistical analysis, and interpretation of results. M.N. and D.T. contributed to preparing the experimental sites and cooperated with data acquisition. Y.M. conceived and designed the study and contributed to the interpretation of results and manuscript preparation. All authors have read and approved the final version submitted for publication.

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Forest Walking Affects Autonomic Nervous Activity: A Population-Based Study

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The present study aimed to evaluate the effect of walking in forest environments on autonomic nervous activity with special reference to its distribution characteristics. Heart rate variability (HRV) of 485 male participants while walking for ~15 min in a forest and an urban area was analyzed. The experimental sites were 57 forests and 57 urban areas across Japan. Parasympathetic and sympathetic indicators [InHF and In(LF/HF), respectively] of HRV were calculated based on ~15-min heart rate recordings. Skewness and kurtosis of the distributions of InHF and In(LF/HF) were almost the same between the two environments, although the means and medians of the indicators differed significantly. Percentages of positive responders [presenting an increase in InHF or a decrease in In(LF/HF) in forest environments] were 65.2 and 67.0%, respectively. The percentage of InHF was significantly smaller than our previous results on HRV during the viewing of urban or forest landscapes, whereas the percentage of In(LF/HF) was not significantly different. The results suggest that walking in a forest environment has a different effect on autonomic nervous activity than viewing a forest landscape.

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Kobayashi H, Song C, Ikei H, Park B-J, Lee J, Kagawa T and Miyazaki Y (2018) Forest Walking Affects Autonomic Nervous Activity: A Population-Based Study. Front. Public Health 6:278. doi: 10.3389/fpubh.2018.00278 Keywords: forest therapy, walking, heart rate variability (HRV), skewness, kurtosis, population approach

INTRODUCTION

"Shinrin-yoku" is a Japanese term for "forest bathing," which was coined by the Director of the Japanese Forestry Agency, Tomohide Akiyama, in 1982 (1). This term is now increasingly being used internationally (1-3). Various studies on the psychological effects of natural environments have been conducted, with consistent effects of reducing negative emotions, such as anger, fatigue, or sadness, being demonstrated in previous studies (4). In addition to psychological effects, beneficial effects of a forest environment in terms of physiological responses have also been investigated (5). Decreases in blood pressure (6–8), in serum or salivary cortisol concentration (6, 9, 10), and enhancements in immune system functioning (11–13) have been reported.

Heart rate variability (HRV) measurement is a method for evaluating autonomic nervous functions. HRV measurement is the most frequently used physiological indicator in studies on the effect of forest environments and demonstrates better results than other physiological measurements, such as salivary cortisol concentration (10). The power spectrum of the heartbeat interval sequence generally exhibits two spectral components: a high-frequency (HF; 0.15–0.40 Hz) component and a low-frequency (LF; 0.04–0.15 Hz) component. The HF component of HRV is

considered to be a marker of parasympathetic activity, whereas the LF component or LF/HF ratio is considered to be a marker of sympathetic activity (14, 15). Several studies have consistently demonstrated increases in HF and/or decreases in LF/HF in forest environments compared with the corresponding levels in urban environments (16–18). These results suggest that being present in a forest environment relaxes the autonomic nervous system.

HRV measurements have the advantages of enabling continuous ambulatory monitoring and robustness against artifacts, such as body movement. These advantages might be maximized in measurement performed during walking in a field environment rather than during resting in laboratory condition. HRV measurements have also been applied in studies on the effects of walking in natural environments (19–21), which also reported relaxation of autonomic nervous system in forest environments similar to that in studies conducted on a resting condition. The present study investigated the HRV of 485 young male participants during walking in forest and urban environments.

In efforts to promote human health, there are two types of strategy: a high-risk (individual) approach and a population approach. The high-risk approach targets individuals with a certain disease or impairment, whereas the population approach targets an entire population. Nature therapy, including "shinrinvoku," is one of the population approaches to promote health. Although its effects on each individual are relatively small, at the population level, it can achieve greater health improvement by shifting the risk distribution curve of the entire population (22). Thus, the beneficial effect of exposure to the natural environment should be evaluated using a population-based analysis with special reference to its distribution characteristics. However, most previous studies on nature therapy have merely focused on the change in the mean values of health-related variables [e.g., (6-13)]. To adopt a population-level perspective, in this study, we analyzed HRV indicators of 485 male participants with special reference to their distribution characteristics. In addition, we compared our results obtained during walking with those during the viewing landscapes reported in a previous study (17).

MATERIALS AND METHODS

Study Sites and Participants

The study areas were 57 forests and 57 urban sites across Japan. Urban areas were downtown or near a Japan railway station. Although 684 young (aged 19–29 years) Japanese male university students participated in the experiments, only 520 participants whose complete data could be obtained at both forests and urban sites were analyzed. Demographic parameters of the participants are shown in **Table 1**. None of the participants reported a history of physical or psychiatric disorders. During the study period, alcohol and tobacco consumption was prohibited and caffeine consumption was controlled.

Experimental Design

The experiment was performed at each experimental area over 2 consecutive days. Prior to the experiment, the aim of this

	Age (year)	Height (m)	Body mass (kg)
Max	29	1.88	110
Min	19	1.55	42
Mean	21.7	1.72	64.6
SD	1.6	0.06	9.5

TABLE 1 | Demographics of the participants (n = 520).

SD, standard deviation.

study and the experimental protocol was explained and general instructions were provided to the participants. The participants participating in an experiment at each site were randomly divided into two groups, and the order of the experimental conditions (urban or forest) was counterbalanced among them. One group performed the experiment in the forest area prior to the urban area, and the other group performed the same experiment in the urban area prior to the forest area. All participants stayed in a waiting room before moving to the field site. All participants were instructed to rest in a chair for \sim 5 min, which mitigated the physiological effects of physical activity before the measurement period. The HRV data were obtained during walking in a forest or an urban environment for $\sim 15 \text{ min.}$ On the second day, the participants switched field sites. The experimental protocol for the second day was the same as the first day.

Among the experiments at 57 locations, those at 44 locations were performed with the experimental design of "Stay-in Forest Therapy," in which all participants were instructed to reside in a hotel with identical single rooms. Meanwhile, the experiments at 13 locations were performed with the experimental design of "One-Day Forest Therapy," in which the participants returned home after the first day of the experiment. To reduce the burden on participants and the research expenses, eventually all experiments were switched to the simplified experimental design of One-Day Forest Therapy.

HRV Measurements

HRV was measured using a portable electrocardiograph (Activtracer AC-301A; GMS, Japan). Spectral analyses of HRV in 15-min recordings were conducted using HRV software (MemCalc/Win; GMS, Tokyo, Japan) based on the maximum entropy method. HF and LF components were obtained by integrating the power spectra at their respective ranges of 0.15–0.40 and 0.04–0.15 Hz. The natural logarithms of the HRV indices [InHF, In(LF/HF)] were then calculated because it has been reported that the raw HRV components exhibit skewed distributions (23).

In this study, HRV was measured during spontaneous breathing, and paced breathing was not applied. The participants were instructed to avoid irregular breathing during the measurements. A previous study reported that the effect of paced breathing on inter-individual variations in the spectral components of HRV was negligible (24).

Outlier Processing

Outlier processing was performed on the results because higher-moment statistics (skewness and kurtosis) are particularly sensitive to outliers (25). The outlier processing was based on a box-whisker plot (26). Upper and lower cut-offs (upperCO and lowerCO, respectively) were defined as follows:

UpperCO = Q3 + 1.5 (IQR), (1) LowerCO = Q1 - 1.5 (IQR), (2)

where

Q1: quartile 1 (25th percentile) Q3: quartile 3 (75th percentile) IQR: interquartile range (Q3–Q1)

The outlier processing was performed on the HRV indices [lnHF and ln(LF/HF)] obtained in each environment (urban and forest). The lowerCOs and upperCOs are summarized in **Table 2**.

The participants associated with outliers in either environment were eliminated. As a result, 35 participants were eliminated, and the data of the remaining 485 participants were used for further analysis.

Statistical Analysis

HRV indicators of the 485 participants were plotted as histograms by dividing the range [from 1.0 to 7.5 for lnHF, from 0.0 to 4.0 for ln(LF/HF)] into 40 segments. Changes in HRV indices between urban and forest environments (forest–urban) were also plotted as a histogram by dividing the range [from -4.5 to +5.5 for lnHF, from -3.0 to +2.0 for ln(LF/HF)] into 40 segments.

The mean, median, standard deviation (SD), coefficient of variation (CV), IQR, skewness, and kurtosis of the distribution were calculated. Skewness is a measure of the symmetry of distribution. Negative or positive skewness is indicated when the left or right tail, respectively, of the research data in a histogram is longer than the other tail. The skewness of a normal distribution is zero. Meanwhile, kurtosis is a measure of whether the distribution curve is peaked (positive) or flat (negative) relative to the normal distribution. The kurtosis of normally distributed data is defined as zero.

Differences in these statistics between urban and forest environments were tested by performing a permutation test, which is a statistical test with a non-parametric basis. Resampling was performed 5,000 times. The *p*-value was calculated according to the suggestion by Phipson and Smyth (27). The uncertainty of a *p*-value near 0.05 was estimated to be 0.3%.

	InHF		In(LF/HF)		
	Urban	Forest	Urban	Forest	
LowerCO	0.85	1.09	0.49	0.22	
UpperCO	6.95	7.54	3.83	3.76	

CO, Cut-off value for the outlier processing.

For further analysis, results of this study were compared with those of our previous study (17). In the previous study, autonomic responses to urban and forest environments were studied in 625 young male participants. The participants viewed the landscape (forest or urban environment) for 15 min while sitting on a chair. When viewing the landscapes, HRV was monitored continuously.

Number of participants who indicated positive or negative responses were calculated for present (walking) and the previous (viewing) results. Positive and negative responses to forest environments were defined as a decrease in lnHF and an increase in ln(LF/HF), respectively. The difference between the present and previous studies with respect to the ratio of negative/positive responders was compared using Chi-squared test. *p*-values < 0.05 were considered indicative of statistical significance for permutation and Chi-squared tests.

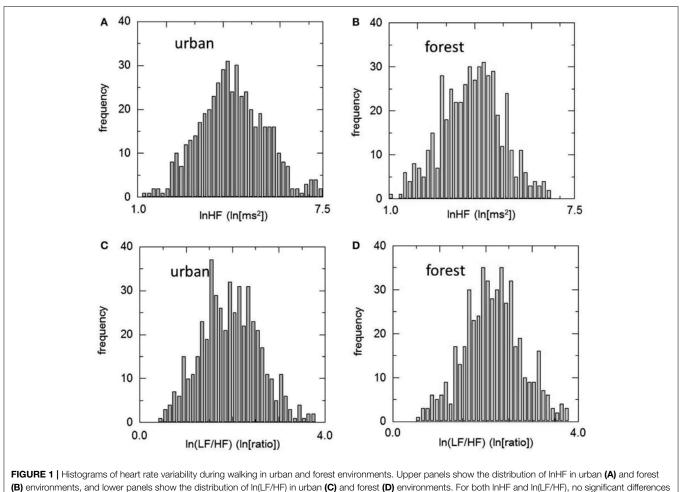
ETHICAL CONSIDERATIONS

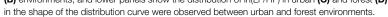
The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Forestry and Forest Products Research Institute, Japan (project identification code number: 16-558), or the Center for Environment, Health and Field Sciences, Chiba University, Japan (project identification code number: 5). Participants were informed about the purposes and procedures of the study and provided written informed consent prior to enrollment. They were free to not attend or cease participation in the program at any time.

RESULTS

Histograms of HRV indicators during walking in urban and forest environments are shown in **Figure 1**, and statistics of these indicators are summarized in **Table 3**. The means of lnHF were 3.93 and 4.33 for the urban and forest environments, respectively. The permutation test revealed that mean lnHF during walking in a forest was significantly larger than during walking in an urban area (p < 0.01). The medians of lnHF were 3.96 and 4.27 for the urban and forest environments, respectively, which were also significantly different (p < 0.01). Although the difference was not significant (p = 0.06), SD was slightly greater in the forest environment than in the urban environment, resulting in CV being almost unchanged (p = 0.83). Both Q1 and Q3 were larger in forest walking (p < 0.01), and as a result, there was no difference in IQR (p = 0.62).

In regards to In(LF/HF) the means were 2.16 in the urban environment and 1.96 in the forest environment, and significantly larger In(LF/HF) was observed in the urban environment than in the forest environment (p < 0.01). As for the median of Q1 and Q3, the differences between urban and forest areas were statistically significant, but the differences in SD and IQR were not as significant. These results were similar to those





of lnHF, although the direction of the change was the opposite. Unlike the results of lnHF, nevertheless, CV of ln(LF/HF) was significantly larger in the forest environment (32.1) than in the urban environment (28.5) (p < 0.01).

The mean and median values were very close in both HRV indicators and in both environments. For example, the values were 3.93 (mean) and 3.96 (median) for lnHF in an urban area. This suggested that the distribution curves of this variable were almost symmetric. This symmetrical distribution was also confirmed by higher moment statistics. Skewness and kurtosis were close to zero for both HRV indicators and both environments, suggesting nearly normal distributions.

The differences between urban and forest environments for lnHF and ln(LF/HF) were plotted in a histogram (**Figure 2**). Positive and negative values in the abscissa represent increases and decreases in the HRV indicator in a forest environment, respectively. Due to an increase in lnHF or a decrease in ln(LF/HF) is considered to represent relaxation, it was defined that these changes are positive responses. Conversely, a decrease in lnHF and an increase in ln(LF/HF) were defined

as negative responses. As for lnHF, 316 (65.2%) participants showed positive responses in the forest environment rather than in the urban environment, and the remaining 169 (34.8%) participants exhibited negative responses. The ln(LF/HF), 325 (67.0%) showed decreases in the forest environment and the remaining 160 (33.0%) exhibited negative responses.

The present results of HRV during walking were compared with the previously reported results on HRV during the viewing of landscapes (17). The numbers of participants who indicated positive/negative responses in HRV indicators in a forest are summarized in **Table 4**. In our previous results (17), 79.2% participants exhibited positive responses (increases in the forest environment) in lnHF during the viewing of landscapes. The proportion of positive responders during viewing was considerably larger than the proportion during walking. A chisquare test revealed significant difference in the proportion of positive responders in lnHF between walking and viewing (p < 0.01).

On the other hand, the proportion of positive responders in ln(LF/HF) during viewing was 64.0%, which was close to the proportion during walking (67.0%) demonstrated in this study.

	InHF			ln(LF/HF)		
	Urban	Forest	Difference (p-value)	Urban	Forest	Difference (p-value)
Mean	3.93	4.33	p < 0.01	2.16	1.96	<i>p</i> < 0.01
Median	3.96	4.27	p < 0.01	2.18	1.95	<i>p</i> < 0.01
SD	1.06	1.16	p = 0.06	0.62	0.63	p = 0.55
CV (%)	27.0	26.74	p = 0.83	28.5	32.1	p < 0.01
Q1	3.17	3.56	<i>p</i> < 0.01	1.74	1.53	<i>p</i> < 0.01
Q3	4.65	5.11	p < 0.01	2.54	2.39	<i>p</i> < 0.01
IQR	1.49	1.55	p = 0.62	0.80	0.86	p = 0.34
Skewness	-0.03	0.13	p = 0.24	0.10	0.18	p = 0.47
Kurtosis	-0.30	-0.13	p = 0.43	-0.15	-0.24	p = 0.64

TABLE 3 | Distribution characteristics of heart rate variability indices urban and forest environments.

SD, standard deviation; CV, coefficient of variation; Q1, quartile 1 (25th percentile); Q3, quartile 3 (75th percentile); IQR, interquartile range; Skewness, a measure of symmetry of distribution; Kurtosis, a measure of whether the distribution curve is peaked (positive) or flat (negative) relative to the normal distribution. Differences between urban and forest environments were tested by a permutation test.

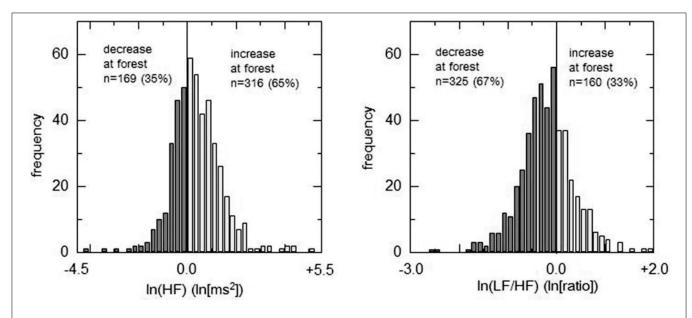


FIGURE 2 | Histograms of difference in heart rate variability indicators between urban and forest environments. Left and right panels demonstrate histograms for the difference in InHF and ln(LF/HF), respectively. As for the parasympathetic indicator (InHF), the percentage of positive responders (presenting an increase in forest environment) was ~65%. Regarding the sympathetic indicator [ln(LF/HF)], the percentage of positive responders (presenting a decrease in forest environment) was ~67%.

A chi-square test revealed that this difference in ln(LF/HF) was not statistically significant (p = 0.30).

DISCUSSION

Analysis of Distribution Characteristics

One of this study's feature is the inclusion of an analysis with special reference to the distribution characteristics of individual variations in the HRV response. Skewness and kurtosis of HRV indices did not change in either lnHF or ln(LF/HF), although significant changes in the mean values were observed between urban and forest environments. In other words, walking in a

forest environment shifted the distribution curve higher (lnHF) or lower [ln(LF/HF)] while maintaining its shape. This was similar to the results in HRV during the viewing of urban and forest landscapes in a previously reported study (17).

Not all physiological indicators, however, maintain the shape of their distribution curve in response to natural environments. Salivary cortisol concentration indicated a significant decrease in forest environments compared with that in urban environments, accompanying a more skewed and kurtotic distribution (10). This modification of the distribution curve might be attributed to a floor effect (28, 29). Therefore, an unchanged distribution curve is a specific response in log-transformed HRV indicators. **TABLE 4** | Number of participants who indicated positive / negative response of HRV indices in forest environment.

	InHF		In(LF/HF)	
	Positive response	Negative response	Positive response	Negative response
Walking ($n = 485$)	316 (65.2%)	169 (34.8%)	325 (67.0%)	160 (33.0%)
Viewing* ($n = 625$)	495 (79.2%)	130 (20.8%)	400 (64.0%)	225 (36.0%)
Chi-squared	27.4 (p < 0.01)		1.0 (p = 0.30)	

*Results on HRV during viewing urban or forest landscapes were presented in our previous report (17).

Effects of Natural Environment on HRV

During walking in forest environments, larger lnHF and smaller ln(LF/HF) were observed compared with those upon walking in urban environments. As the lnHF and ln(LF/HF) are indicators of parasympathetic and sympathetic nervous activity, the present results implied that the autonomic relaxation occurred during walking in forest environments. The results are consistent to those in our previous study (17). Therefore, walking in forest environments and viewing forest landscapes demonstrated qualitatively similar effects on autonomic functions.

Controversy, quantitative comparisons between the present and previous results revealed a different tendency in the autonomic response to walking and viewing. During walking in forest environments, 65.2% participants exhibited a positive response in the parasympathetic indicator (lnHF), which was significantly lower than the percentage of positive responders during viewing of forest landscape (79.2%). Contrary, for the sympathetic indicator, the percentage of positive responders during walking (67.0%) was almost identical to that during viewing (64.0%). Therefore, the effect of a forest environment on parasympathetic nervous activity was more apparent during viewing than walking, whereas sympathetic activity exhibited almost the same responses to viewing and walking regarding the percentage of positive responders.

Positive and Negative Effects of a Natural Environment

In 1984, the distinguished biologist Edward O. Wilson proposed the biophilia hypothesis (30). Biophilia is defined as the "innate tendency to focus on life and life-like processes" (31). For millions of years, our ancestors lived in the savannas of Africa. Within this environment, natural features, such as trees or forests, could provide food, water, or shelter, thereby increasing the probability of survival. Thus, biophilia can be regarded as an adaptive characteristic.

Alternatively, it is known that certain people show a strong dislike for natural settings. This tendency is called biophobia (32). Biophobia includes certain specific phobias, such as arachnophobia (irrational fear of spiders) or entomophobia (fear of insects). There is also a term referring to the fear of forests (hylophobia/xylophobia) (33). Biophobia is also an

adaptive psychological trait because of inherent dangers in the natural environment (e.g., predators and poisonous organisms). Therefore, the effect of the natural environment on humans is two-sided.

From the perspective of evolutionary psychology, a model for the effects of the natural environment on humans has been proposed, which includes three factors: drive, contentment, and threat (34, 35). Drive includes emotions such as joy, approach, appetite, stimulation, and positiveness. As an endocrine response, it is related to dopamine secretion. In contrast, contentment is concerned with emotions such as calmness, relaxation, and safety and is related to the oxytocin and opiate systems. In terms of autonomic regulation, drive and contentment are associated with sympathetic and parasympathetic activities, respectively (35). A relaxation in autonomic nervous activity [increase in lnHF and decrease in ln(LF/HF)] was observed in the forest environment during both walking and viewing; therefore, it can be considered that exposure to a forest environment mainly confers contentment rather than drive. Furthermore, a comparison between present and our previous results suggested that viewing a forest landscape could provide more contentment than walking in a forest environment.

A major limitation of this study is that it included only Japanese young male subjects. The tendency for biophilia/biophobia may be affected by difference in age, gender, and ethnicity of participants. Effects of demographic and geographic factors on physiological responses to natural environments should be investigated in a future study.

CONCLUSION

The autonomic relaxation (increases in parasympathetic indicator and/or decreases in sympathetic indicator) in forest environments has been demonstrated by HRV analysis in previous studies. This result was also confirmed in this study. However, a comparison between the present and our previous study (17) suggested that the response of HRV differ between viewing and walking.

The effect of forest environments consists of several factors, including negative emotions. It is reasonable that a certain percentage of a population exhibits a negative response to forest environments. Therefore, population-based analysis is required in which the existence of negative responders is taken into consideration.

AUTHOR CONTRIBUTIONS

HK contributed to statistical analysis, interpretation of the results, and manuscript preparation. CS and HI were involved with data acquisition and initial analysis of the results. B-JP, JL, and TK participated in data acquisition and study design. YM had an important role in the research, particularly in experimental design, data acquisition, and manuscript preparation. All authors contributed to the preparation of the manuscript and are responsible for the final editing and approval.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Article Psychological Benefits of Walking through Forest Areas

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Abstract: This study aimed to clarify the psychological benefits of brief walks through forest areas. In addition, we aimed to examine the associations between psychological responses and trait anxiety levels. Five-hundred-and-eighty-five participants (mean age, 21.7 ± 1.6 years) were instructed to walk predetermined courses through forest (test) and city (control) areas for 15 min. The Profile of Mood State (POMS) questionnaire and State-Trait Anxiety Inventory were used to assess participants' psychological responses and trait anxiety levels, respectively. The results revealed that walking through forest areas decreased the negative moods of "depression-dejection", "tension-anxiety", "anger-hostility", "fatigue", and "confusion" and improved the participants' positive mood of "vigor" compared with walking through city areas. Furthermore, a significant correlation was found between participants' trait anxiety levels and their changes in the subscale of "depression-dejection" of POMS after walking through forest areas. A more effective reduction in the feeling of "depression-dejection" after walking through forest areas areas and subserved for participants with high trait anxiety levels than for those with normal and low trait anxiety levels. This study showed the psychological benefits of walking through forest areas and identified a significant correlation between psychological responses to walking through forests and trait anxiety levels.

Keywords: forests; shinrin-yoku; forest therapy; psychological relaxation; profile of mood state; brief walks; individual difference; trait anxiety

1. Introduction

As individuals are exposed to several stressors in daily life, they attempt to adopt effective methods for coping with stress and for relaxing. Nature-based experiences have relaxing effects, and the therapeutic effect of nature has received increasing attention.

The positive effects of nature on physical and mental health have been recently reported [1–4], and the concept of natural therapy has been developed. Nature therapy is defined as "a set of practices aimed at achieving 'preventive medical effects' through exposure to natural stimuli that render a state of physiological relaxation and boost the weakened immune functions to prevent diseases" [2]. Nature therapy seeks to improve immune functions, prevent illnesses, and maintain and promote health through exposures to nature with consequent attainment of a relaxed state [1,2].

Numerous studies have focused on and demonstrated the effects of forests in mitigating stress states and inducing physiological relaxation [5–12]. Spending time in forests improves immune functions [13,14], and these effects last for approximately 1 month [15]. In addition, experiments with elderly individuals and adults who are at a risk for stress- and lifestyle-related diseases such as high blood pressure, diabetes, and depression found that various activities performed in forests have positive effects [16–24].

However, these studies were limited to small sample sizes, and individual differences within these effects have been noted. A previous study revealed the individual differences in changes of blood pressure after walking through forests [25]. Although some participants showed a decrease in blood pressure after walking through forests, others showed an increase. People respond differently even to the same stimuli. Examination of these individual differences using their initial values demonstrated that participants with initial high blood pressures showed a decrease in blood pressures after walking in a forest, whereas those with initial low blood pressures showed an increase; it concluded that exposure to forest environments had a physiological adjustment effect close to an appropriate level [25]. Moreover, one study [26] assessed individual differences using type A behavioral patterns [27,28], which are known to be specific behavior patterns often exhibited by patients with a heart disease. A previous experiment [26] that involved viewing both forest and city landscapes for 15 min compared the change in the participant's pulse rate. The results revealed that although the pulse rate decreased in the forest compared with that in the city in the group with type B behavior pattern, which is opposite to the type A behavior pattern. However, there was no significant change in the pulse rate in the group with type A behavior pattern. These results show that physiological responses can vary depending on various factors such as initial values and behavioral patterns [25,26].

Regarding psychological aspects, the restorative effects of natural environment, including forests, that are associated with psychological stressors or mental fatigue, decreased depressive symptoms, and improved mood states have been reported [9,11,29–33]. Kaplan and Kaplan [29] have reported that mental fatigue experienced by individuals nowadays could be restored by contact with a natural environment, and Ulrich et al. [30] demonstrated that natural scenery relieves psychological stress. Shin et al. [31] assessed the effect of forest environment on an individual's psychological health and well-being and the contribution of a forest experience to improved emotional and cognitive health. In relation to the more direct impacts of natural environment in humans, Park et al. [32] showed that walking around and viewing forests improved emotional state, such as tension and anxiety, depression and dejection, anger and hostility, vigor, confusion, and fatigue, leading to psychological relaxation. Morita et al. [33] reported that walking and staying in forests decrease feelings of hostility and depression and increase liveliness.

Individual differences also exist with regard to psychological responses, and this phenomenon requires further clarification. We previously examined individual differences in changes of mood states such as "depression-dejection", "tension-anxiety", "anger-hostility", "fatigue", "confusion", and "vigor" after walking and viewing forests and found significant correlations between them and participants' initial values [34]. However, studies regarding individual differences in psychological responses are insufficient.

With a large sample population, this study aimed to clarify the psychological benefits of brief walks through forest areas. In addition, we assessed the associations among changes in the mood state of "depression-dejection" after walking through forest areas and trait anxiety levels because mental health problems such as depression and high anxiety are common in modern societies.

2. Materials and Methods

2.1. Participants and Experimental Sites

From 2005 to 2013, we performed experiments in 52 forest and city areas of Japan. Figure 1 presents a map showing the distribution of all 52 locations. Experiments were conducted in representative

forests in each region, and safe, well-maintained forest areas were selected as the experimental sites. City areas were either downtown or near a Japan Railway station. All experiments were conducted during summer between July and September.

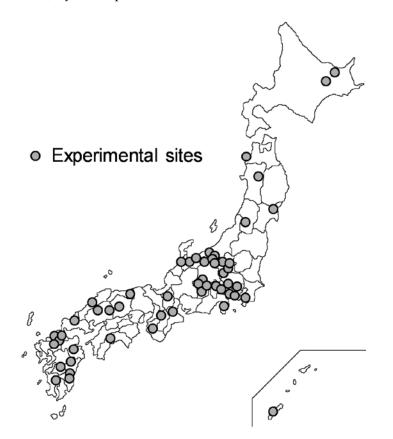


Figure 1. A map showing the distribution of all 52 locations.

Twelve male Japanese university students participated in each experiment (N = 624 participants; 12 participants \times 52 areas), and no student reported a history of physical or psychiatric disorder. Of the 624 participants, data from 585 participants (mean age, 21.7 \pm 1.6 years) were analyzed. The demographic parameters of the participants are given in Table 1. During the study period, alcohol and tobacco consumption was prohibited, and caffeine consumption was controlled. The study was performed with the approval of the Institutional Ethics Committee of the Forestry and Forest Products Research Institute (project identification code number: 16-558; from 2005 to 2006; 19 areas with 228 participants) and the Ethics Committee of the Center for Environment, Health and Field Sciences, Chiba University (project identification code number: 5; from 2007 to 2013; 33 areas with 396 participants) in Japan.

Parameter	Mean \pm Standard Deviation
Total sample number	585
Age (years)	21.7 ± 1.6
Height (cm)	172.4 ± 5.6
Weight (kg)	64.6 ± 9.4
BMI ¹ (kg/m ²)	21.7 ± 2.9

¹ BMI, body mass index.

2.2. Experimental Design

Twelve participants visited the orientation site in each experimental region on the day before (39 areas) or the morning of (13 areas) the experiment. Before initiating the experiments, we explained the aims and procedures of the study to all participants and obtained their written informed consent. Participants were randomly divided into two groups of six persons. On the first day, one group performed the experiment in the forest area, and the other performed the same experiment in the city area. On the second day, participants switched field sites to eliminate order effects. On arrival in the forest or city area, the participants awaited their turn in a waiting room and were eventually taken, one by one, to the experimental site. Each participant took a walk along the given course for approximately 15 min (Figure 2).

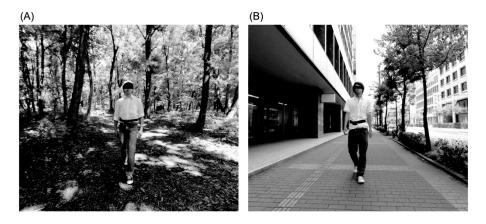


Figure 2. Experimental scenery. (A) Forest area and (B) city area.

2.3. Psychological Measurements

For evaluated participants' mood state, the Profile of Mood State (POMS) questionnaire was used. POMS is a well-established, factor analytically-derived measure of psychological distress, and its high reliability and validity levels have been previously documented [35,36]. POMS simultaneously evaluates six moods: depression and dejection (D), tension and anxiety (T-A), anger and hostility (A-H), fatigue (F), confusion (C), and vigor (V). We used T-scores of POMS for the analysis. In this study, we used the Japanese version of POMS and a short form with 30 questions [37] to reduce the burden on participants. The evaluations of POMS were conducted before and after walking in two areas.

In addition, the State-Trait Anxiety Inventory (STAI) form JYZ was used to assess the participants' trait anxiety level. STAI is a self-reported tool that measures the presence and severity of current symptoms of anxiety and a generalized propensity to be anxious [38]. Unlike state anxiety, which is a measure of the current state of anxiety that assesses how respondents feel "right now", trait anxiety is a measure of the relatively stable aspects of "anxiety proneness" as assessed by 20 questions [39]. In our study, scores of \geq 44 were considered to be the high trait anxiety group, scores of \leq 43 and \geq 33 were considered to be the normal trait anxiety group, and scores of \leq 32 were considered to be the low trait anxiety group.

2.4. Data Analysis

The Wilcoxon signed-rank test was used to compare psychological responses after walking through the forest and city areas.

Pearson's correlation test was used to analyze the correlation between scores of the POMS subscales after walking through forest areas (the value after walking through a forest area compared to the value after walking through a city area) and those of trait anxiety of STAI.

Mann–Whitney U test was used to between participants with high trait anxiety levels and those with normal and low trait anxiety levels.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 20.0, SPSS Inc., Chicago, IL, USA). In all cases, *p*-values of <0.05 were considered to be statistically significant.

3. Results

Significant differences between walking through forest and city areas were observed for all subscales of D, T-A, A-H, F, C, and V (Figure 3). The score of the D subscale was 40.6 ± 3.7 (mean \pm standard deviation) after walking through forest areas, which was significantly lower than 41.6 ± 5.3 after walking through city areas (p < 0.01). Similar results were obtained for T-A (forest, 35.5 ± 5.5 ; city, 40.4 ± 7.7 ; p < 0.01), A-H (forest, 38.1 ± 3.9 ; city, 39.5 ± 4.7 ; p < 0.01), F (forest, 37.5 ± 6.2 ; city, 42.7 ± 8.4 ; p < 0.01), and C (forest, 39.8 ± 5.6 ; city, 42.2 ± 6.7 ; p < 0.01) subscales, and a decrease in negative mood state was observed after walking through forest areas was 42.6 ± 10.4 , which was significantly higher than 35.1 ± 8.9 after walking through city areas (p < 0.01); thus, an increase in positive mood state was observed after walking through forest areas.

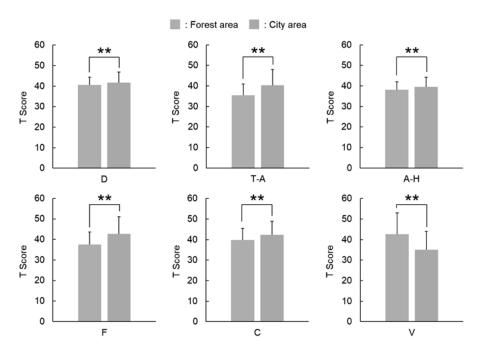


Figure 3. Scores of the Profile of Mood States after walking through forest and city areas. (D), depression-dejection; (T-A), tension–anxiety; (A-H), anger-hostility; (F), fatigue; (C), confusion; and (V), vigor. N = 585; mean \pm standard deviation; **, p < 0.01 by Wilcoxon signed-rank test.

Figure 4 shows a three-dimensional graph in which the *x*-axis denotes the changes after following walking through forest areas, the *y*-axis denotes the trait anxiety scores of STAI, and the *z*-axis denotes the number of participants. A significant correlation was observed between changes in the D subscale after walking through forest areas (the value after walking through a forest area compared to the value after walking through a city area) and the participants' trait anxiety levels (p < 0.01; Figure 4).

Participants with high trait anxiety levels tended to have a more effective reduction in the feeling of "depression-dejection" after walking through forest areas compared with those with normal and low trait anxiety levels (participants with high trait anxiety, N = 327; participants with normal and low trait anxiety, N = 258; p = 0.075).

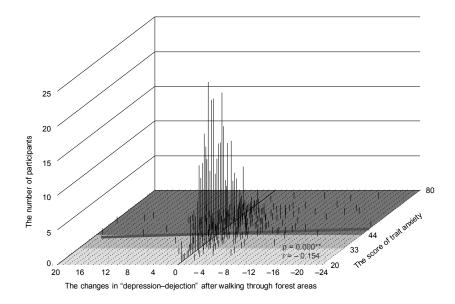


Figure 4. Three-dimensional graph showing the changes in "depression-dejection" after walking through forest areas, trait anxiety scores, and number of participants. N = 585, **: p < 0.01 by Pearson's correlation test.

Of the 585 participants, 182 participants showed decreased feeling of "depression-dejection" after walking in forests. Meanwhile, 56 participants experienced increased feeling of "depression-dejection," and 347 participants did not experience any changes. Figure 5 shows the results of participants whose feelings of "depression-dejection" decreased after walking through forest areas. A significant correlation was observed between changes after walking through forest areas and the participants' trait anxiety levels (p < 0.01; Figure 5).

Participants with higher trait anxiety levels tended to show greater decreases than those with normal and low trait anxiety levels (participants with high trait anxiety, N = 123; participants with normal and low trait anxiety, N = 59; p = 0.064).

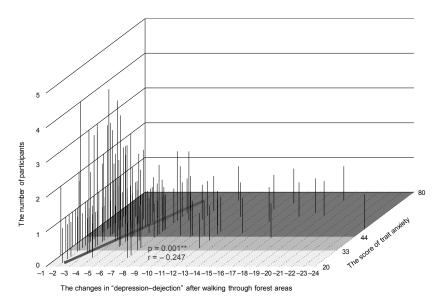


Figure 5. Three-dimensional graph on the changes in "depression-dejection" after walking through forest areas, trait anxiety score, and number of participants in the decreasing group. N = 182; **, p < 0.01 by Pearson's correlation test.

4. Discussion

This study found that walking through forest areas decreased the negative moods of "depression-dejection", "tension-anxiety", "anger-hostility", "fatigue", and "confusion" and improved the participants' positive mood of "vigor" compared with walking through city areas. These results, which demonstrate the psychological benefits of forests, are partly consistent with previous findings of the effect of viewing forest scenery or walking in forests [9,11,32]. Park et al. [32] has demonstrated that walking around and viewing forests improved negative emotions, such as depression-dejection, tension-anxiety, anger-hostility, fatigue, and confusion, as well as a positive emotion of vigor, in 168 participants at 14 locations; these results are consistent with our findings. Present study is the first study to use a sample size as large as 585 participants, and the psychological benefits of walking through forests were evident with this larger sample.

A significant correlation was found between participants' trait anxiety levels and their changes in the "depression-dejection" subscale of POMS after walking through forest areas. Our data revealed that psychological responses can differ depending on a participant's trait anxiety levels and that those participants with high trait anxiety levels tended to have a more effective reduction in the feeling of "depression-dejection" after walking through forest areas than participants with normal and low trait anxiety levels. Only the feeling of "depression-dejection" had a significant correlation, and no significant correlation was found between the other subscales of POMS. In future studies, this point must be considered. Very few studies have assessed individual differences in psychological responses, and therefore, more researches in this area are required.

More than half of the global population currently lives in urban environments, and 69% of individuals are expected to live in urban areas by 2050 [40,41]. Although urbanization has led to improvements in many areas such as housing, employment, education, equality, quality of living environment, social support, and health services [42], changes that have occurred over a very short period have been very drastic from an evolutionary perspective. Recent research showed that city dwellers are constantly exposed to stressors and that urban living is associated with increased risk of health problems [43–46]. In particular, mental health problems were profound. Current city dwellers have a 39% higher risk for mood disorders and 21% higher risk for anxiety disorders [44] and higher rates of psychotropic medication prescriptions for anxiety, depression, and psychosis [46]. Therefore, the psychological benefits of walking through forests are very significant, and forest environments are expected to have very important roles in promoting mental health in the future. It is necessary to consider the health policy using nature including forests. Furthermore, urban planners should pay more attention to maintaining and increasing accessible greenery in urban areas. The beneficial effects of nature suggest a simple, accessible, and cost-effective method to improve the quality of life and health of urban residents.

This study had several limitations. Firstly, this study was conducted in representative forests in each region to validate the psychological effect of walking in forest areas. Because the experiments were conducted at 52 different sites, the difference according to region might have affected the result. The effects according to the various characteristics of the forests must be examined in the future. Secondly, to generalize the findings, further studies that include various other demographic groups such as females and individuals with different ages are required. Thirdly, for an overall discussion, verifying the effect of forests using other psychological measurements is necessary to demonstrate the psychological effect of forests. Finally, participants' prior expectations and experience with forests may influence the results. These limitations should be considered in future research.

5. Conclusions

This study demonstrated the psychological benefits of walking through forest areas and revealed a significant correlation between psychological responses and trait anxiety levels.

Author Contributions: C.S. performed data acquisition, statistical analysis, interpretation of the results, and manuscript preparation. H.I., B.-J.P., and J.L. were involved with the acquisition and interpretation of data. T.K. designed the study and was involved with data acquisition. Y.M. had an important a role in the overall performance of this research, particularly experimental design, data acquisition, and manuscript preparation. All authors contributed to the preparation and are responsible for the final editing and approval of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Article

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Physiological and Psychological Effects of Forest and Urban Sounds Using High-Resolution Sound Sources

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Abstract: Exposure to natural sounds is known to induce feelings of relaxation; however, only few studies have provided scientific evidence on its physiological effects. This study examined prefrontal cortex and autonomic nervous activities in response to forest sound. A total of 29 female university students (mean age 22.3 ± 2.1 years) were exposed to high-resolution sounds of a forest or city for 60 s, using headphones. Oxyhemoglobin (oxy-Hb) concentrations in the prefrontal cortex were determined by near-infrared spectroscopy. Heart rate, the high-frequency component of heart rate variability (which reflects parasympathetic nervous activity), and the ratio of low-frequency to high-frequency (LF/HF) components (which reflects sympathetic nervous activity) were measured. Subjective evaluation was performed using the modified semantic differential method and profiles of mood states. Exposure to the forest sound resulted in the following significant differences compared with exposure to city sound: decreased oxy-Hb concentrations in the right prefrontal cortex; decreased ln(LF/HF); decreased heart rate; improved feelings described as "comfortable," "relaxed," and "natural"; and improved mood states. The findings of this study demonstrated that forest-derived auditory stimulation induced physiological and psychological relaxation effects.

Keywords: forest sound; natural sound; physiological relaxation effects; prefrontal cortex activity; autonomic nervous activity; near-infrared spectroscopy; heart rate variability; heart rate; semantic differential method; profile of mood states

1. Introduction

Human physiological functions have adapted to the natural environment over 6–7 million years of human evolution [1,2]. However, since the industrial revolution, an increasing number of people have moved from natural to artificial urban environments. According to a United Nations report, 30% of the world's population in 1950 was urban population, and this value is projected to rise to 66% by 2050 [3]. In evolutionary terms, human physiology, which has evolved in response to the natural environment, has had little time to adapt to the artificial environments of urban areas. Some researchers have proposed this to be a reason why many people in urban areas experience stress and tension [2,4,5].

In recent years, the relaxing and restorative effects of the natural environment have gradually gained attention [4–7], with the development of physiological measurement technology facilitating the accumulation of scientific evidence based on physiological parameters.

Field experiment studies, in which all five senses of participants were involved, have reported a physiological relaxation effect of a forest compared with the effect of an urban environment, with findings such as increased parasympathetic nervous activity, decreased sympathetic nervous activity, and decreased cortisol levels and cerebral blood flow in the prefrontal cortex [8–16]. Moreover, residents who lived near a large amount of greenery were shown to have lower chronic stress levels than those who did not [17,18].

In laboratory experiments involving sensory stimuli, the relaxation effect of nature-derived stimulation has been reported. For example, studies have shown positive physiological effects when participants viewed images of a forest landscape [19], wooden materials [20], indoor plants [21–24], and flowers [25,26], which induced increased parasympathetic nervous activity, decreased sympathetic nervous activity, and decreased prefrontal cortex activity. Stress recovery is also faster in people who view natural scenes than in people who view urban scenes [27]. A number of studies have shown that viewing an image of nature through videos and virtual nature scenes results in decreased stress and increased positive emotions [28–30]. Relaxation has also been demonstrated with exposure to olfactory stimuli such as the scent of wood [31,32], fresh roses [33], and rose and orange essential oils [34]. The researchers demonstrated that these olfactory stimuli resulted in increases in parasympathetic nervous activity and decreases in prefrontal cortex activity. Tactile contact with wood resulted in similar physiological effects, increasing parasympathetic nervous activity and decreasing sympathetic nervous activity and prefrontal cortex activity [35–37].

These studies demonstrated that nature-derived visual, olfactory, and tactile stimuli affect the brain and autonomic nervous activities, with these changes inducing a state of relaxation in humans. However, there have been few studies on nature-derived auditory stimulation. One study showed that natural sounds tended to promote the recovery of skin conductance levels compared with road noise [38]. In another study on stress recovery effects, participants were shown a virtual reality image with or without natural sounds; viewing the image with natural sounds resulted in enhanced parasympathetic nervous activity and improved stress recovery [39]. As yet, no studies have investigated the effects of natural auditory stimuli on the indicators of brain and autonomic nervous activities.

Recently, high-resolution sounds have been increasingly used in Japan. High-resolution sound sources are considered to provide highly realistic natural sounds. Although Oohashi et al. [40] have reported the effects of high-resolution sounds using electroencephalogram (EEG), the physiological effects of high-resolution sounds are rarely reported. This study aimed to compare the physiological effects of auditory stimulation with forest and city sounds using high-resolution sound sources.

2. Materials and Methods

2.1. Participants

The study recruited 29 female university students (mean age \pm standard deviation, 22.3 \pm 2.1 years) via a bulletin board at the university. The following exclusion criteria were applied: poor physical condition; a respiratory illness; menstruation on the day of the experiment; a hearing impairment; and smoking.

All participants were informed about the purpose and experimental procedures of the study and gave their informed consent. The study was conducted in line with the principles of the Declaration of Helsinki, and the protocol was approved by the Ethics Committees of the Center for Environment, Health and Field Sciences, Chiba University, Japan (project identification code number, 36). It was registered in the University Hospital Medical Information Network of Japan (UMIN ID: UMIN000034821).

2.2. Auditory Stimulation

To heighten realism, high-resolution sound sources were used as auditory stimuli. We selected the sound of a murmuring brook in the Togakushi forest in Nagano Prefecture as the forest auditory stimulus (forest sound). The other stimulus (city sound) was the sound of city traffic at the Shibuya intersection in Tokyo. The sounds were recorded using a high-resolution sound recorder, with a sample rate of 96 kHz and 24 bit quantization.

The participants' sensory evaluations of sound intensity were scored from 0 to 10: 0, inaudible sound; 2, faint sound; 4, quiet sound; 6, easy to hear sound; 8, loud sound; and 10, very loud sound. It was ensured that there was no significant difference in terms of the subjective sound intensity scores between the forest and city sounds (scores, 6.1 ± 0.1 and 6.0 ± 0.2 , respectively). The auditory stimuli were played to the participants using headphones at 48.6 dB for the forest sound and 51.5 dB for the city sound.

2.3. Study Protocol

After receiving an explanation of the study details and protocol in the waiting room, each participant was moved into a chamber with an artificial climate for physiological measurement (maintained at 25 °C with 50% relative humidity and 200 lux illumination) at the Center for Environment, Health and Field Sciences, Chiba University. This ensured that the participants were exposed to minimal external influences and were tested under the same physical and soundproof conditions. Figure 1 shows the experimental set-up for the physiological measurement upon auditory stimulation, and Figure 2 summarizes the measurement protocol. The physiological measurement devices (heart rate variability (HRV), near-infrared spectroscopy (NIRS)) and headphones that provided auditory stimulation were attached to the participants. The participant was asked to rest, with eyes closed, for one minute, and then the auditory stimulus (forest or city sound) was provided for one minute. The forest and city sounds were provided in a counterbalanced order to eliminate any possible impact of the order on the physiological responses. The physiological activities were measured continuously during the rest and stimulation periods. After measuring the participants' physiological responses, subjective tests were performed for about two minutes.

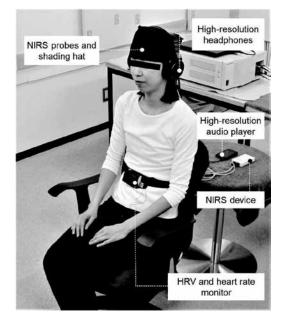


Figure 1. Experimental set-up for auditory physiological measurement. NIRS: near-infrared spectroscopy; HRV: heart rate variability.

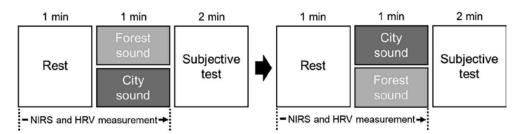


Figure 2. Measurement protocol. The order of the forest and city sounds was counterbalanced to avoid any order effects. NIRS: near-infrared spectroscopy; HRV: heart rate variability.

2.4. Physiological Measurements

2.4.1. Near-Infrared Spectroscopy

When there is an increase in local brain activity, brain blood flow increases, resulting in luxury perfusion such that the brain blood flow exceeds oxygen consumption [41]. This produces a detectable increase in oxyhemoglobin (oxy-Hb) concentration. Changes in oxy-Hb concentrations are known to be consistent with the changes in blood flow in the brain [42], and it is thought that a decrease in oxy-Hb concentration is associated with physiological calming. In this study, near-infrared spectroscopy (NIRS) was used as an indicator of prefrontal cortex activity. Two NIRS probes (Pocket NIRS Duo, Dynasense, Shizuoka, Japan), attached to the left and right forehead, were used to detect changes in the concentrations of oxy-Hb and deoxygenated hemoglobin in the cerebral blood flow [43]. Concentrations in the left and right prefrontal cortex were recorded every second during the rest and auditory stimulation periods. Each data point was then expressed as the difference from the average of the 60 s rest period.

2.4.2. Heart Rate Variability and Heart Rate

Heart rate variability (HRV) and heart rate were used as indicators of autonomic nervous activity [44,45]. Electrocardiography was performed using a portable electrocardiogram (Activtracer AC-301A; GMS, Tokyo, Japan), and HRV was analyzed for the periods between consecutive R waves (RR intervals) in the electrocardiogram. The power levels of the high-frequency (HF; 0.15–0.40 Hz) and low-frequency (LF; 0.04–0.15 Hz) components of HRV were calculated using the maximum entropy method (MemCalc/win; GMS, Tokyo, Japan) [46,47]. The HF component reflects parasympathetic nervous activity, and the LF/HF ratio reflects sympathetic nervous activity [44]. In this study, the natural logarithmic values of HF and the LF/HF power ratio (i.e., ln(HF) and ln(LF/HF), respectively) were used to normalize the participants' HRV values [48]. The average HRV and heart rate values during the rest and stimulation periods were calculated.

Respiratory changes can influence HRV data; therefore, the participants' respiratory rates were estimated during the period between the two stimuli. The respiratory rate can be estimated from the HRV power spectrum [49]. Generally, heart rate accelerates during inspiration and decelerates during expiration [50,51]; thus, the respiratory rate can be estimated from the dominant frequency of the HF component. We calculated the HRV power spectrum using the maximum entropy method and located the maximum power of the HF component using the associated frequency as the dominant respiratory frequency during the measurement period. To detect the peak frequency of the HF component, the model order for spectral analysis was chosen from the 7th to 12th orders, with the 9th order used in principle.

2.5. Psychological Measurements

The participants' psychological feelings associated with each auditory stimulation were evaluated with two subjective tests using questionnaires. The first test used the semantic differential (SD) method [52]; the participant responded to three scales based on opposing adjective pairs (comfortable–uncomfortable, relaxed–aroused, and natural–artificial), each of which was evaluated

on 13 scales. The Profile of Mood States (POMS) questionnaire was used to evaluate mood states from the scores for tension–anxiety (T-A), depression–dejection (D), anger–hostility (A-H), fatigue (F), confusion (C), and vigor (V) [53,54]. The total mood disturbance (TMD) score was calculated using this formula: (T-A) + (D) + (A-H) + (F) + (C) - (V) [54]. This score is practical from a clinical perspective, and is considered highly reliable because of the intercorrelations among the six primary POMS factors [53]. To reduce the burden on the participants, a shortened Japanese version of the POMS with 30 questions [55] was used.

2.6. Statistical Analysis

In the analyses of the physiological indices (NIRS, HRV, heart rate, and respiratory frequency), paired *t*-tests were used to compare the average values for the 60 s auditory stimulation periods between the forest and city sounds. The Wilcoxon signed-rank test was used in the analysis of the psychological measurements to compare the effects of the different stimuli. One-sided tests were used, based on the hypothesis that humans are relaxed by nature-derived auditory stimulation. The statistical package for the social sciences software (version 21.0, IBM, Armonk, NY, USA) was used, and *p*-values < 0.05 were considered statistically significant.

3. Results

3.1. Physiological Effects

3.1.1. Near-Infrared Spectroscopy (NIRS)

Figure 3 shows the time-dependent changes in oxy-Hb concentration per second in the right and left prefrontal cortex during the exposure to the forest and city sounds. In the right prefrontal cortex (Figure 3a), changes in the oxy-Hb concentration were similar for the forest and city sounds for the first 15 s after exposure. From about 16 s, the oxy-Hb concentration was lower after the exposure to the forest sound compared with that after exposure to the city sound. From about 25 s to the end of the exposure period, the oxy-Hb concentration during the exposure to the forest sound remained at a fairly constant level, lower than that during the exposure to the city sound. Oxy-Hb concentrations in the left prefrontal cortex showed similar trends (Figure 3b).

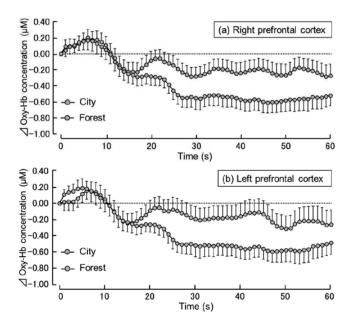


Figure 3. Time-dependent changes in the oxy-hemoglobin (oxy-Hb) concentrations in the right (**a**) and left (**b**) prefrontal cortex during 60 s exposure to the forest vs. city sounds. Data are expressed as mean ± standard error (n = 29).

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Figure 4 shows the comparison of the mean oxy-Hb concentrations during the 60 s exposure to the forest and city sounds. Compared with the exposure to the city sound, the exposure to the forest sound significantly decreased oxy-Hb concentrations in the right prefrontal cortex (forest: $-0.37 \pm 0.03 \mu$ M (mean \pm standard error); city: $-0.14 \pm 0.02 \mu$ M; t(28) = -2.36; p < 0.05; Figure 4a). In the left prefrontal cortex, a decrease was observed in the oxy-Hb concentration during the exposure to the forest sound compared to the exposure to the city sound (forest: $-0.36 \pm 0.03 \mu$ M; city: $-0.14 \pm 0.02 \mu$ M; t(28) = -1.59; p < 0.07; Figure 4b).

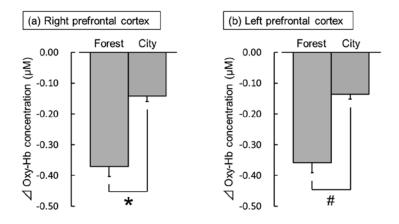


Figure 4. The overall mean oxyhemoglobin (oxy-Hb) concentrations in the right (**a**) and left (**b**) prefrontal cortex during exposure to the forest vs. city sounds. Data are expressed as mean \pm standard error (n = 29). * p < 0.05, # p < 0.07 as determined by paired *t*-test (one sided).

3.1.2. Heart Rate Variability (HRV) and Heart Rate

The HRV data revealed a significant difference in terms of sympathetic nervous activity in response to exposure to the forest and city sounds. Figure 5a shows the overall mean ln(LF/HF) ratio during the 60 s exposure to the forest and city sounds. The ln(LF/HF) ratios were -0.38 ± 0.18 during the exposure to the forest sound and -0.04 ± 0.18 during the exposure to the city sound. This indicates that sympathetic nervous activity was significantly lower during exposure to the forest sound than that during exposure to the city sound (Figure 5a; t(28) = -2.39; p < 0.05). The mean baseline ln(LF/HF) ratios did not differ significantly between the forest (-0.08 ± 0.16) and city (-0.15 ± 0.21) sounds during the 60 s rest period (p > 0.05). The ln(LF) and ln(HF) were 5.28 ± 0.16 and 5.66 ± 0.17 in the forest sound and 5.53 ± 0.14 and 5.57 ± 0.18 in the city sound, respectively. Figure 5b shows the mean heart rate during the 60 s exposure to the two stimuli. The heart rate was 72.2 ± 2.2 bpm during exposure to the forest sound and 72.9 ± 2.3 bpm during exposure to the city sound, i.e., the heart rate was significantly lower during exposure to the forest sound than that during exposure to the city sound (Figure 5b; t(28) = -1.93; p < 0.05). The mean baseline heart rate did not differ significantly between the forest $(73.1 \pm 2.1 \text{ bpm})$ and city $(73.4 \pm 2.3 \text{ bpm})$ sounds during the 60 s rest period (p > 0.05). There was no significant difference in terms of the ln(HF) value between exposure to the two stimuli. Moreover, there was no significant difference in terms of respiratory frequency between the forest and city sounds (0.26 ± 0.01 Hz vs. 0.27 ± 0.01 Hz; t(28) = −1.76; p > 0.05).

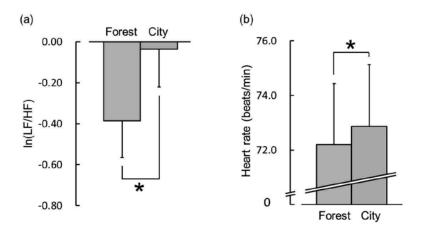


Figure 5. Indicators of sympathetic nervous activity and heart rate during exposure to the forest vs. city sounds for 60 s. (**a**) The overall mean natural log of the low-frequency to high-frequency power ratio of heart rate variability (ln(LF/HF)) and (**b**) overall mean heart rate. Data are expressed as mean \pm standard error (n = 29). * *p* < 0.05 as determined by paired *t*-test (one sided).

3.2. Psychological Effects

3.2.1. Modified Semantic Differential (SD) Method

Figure 6 summarizes the results of the evaluation of the participants' subjective feelings on the three scales, as measured by the modified SD method, after exposure to the forest vs. city sounds. For the comfortable–uncomfortable scale, the mean score was "slightly-to-moderately comfortable" when exposed to the forest sound but was "indifferent-to-slightly uncomfortable" when exposed to the city sound (Figure 6a, p < 0.01). Thus, the exposure to the forest sound induced a more comfortable feeling than exposure to the city sound. For the relaxed–aroused scale, the mean score was "slightly-to-moderately relaxed" with exposure to the forest sound but was "slightly aroused" with exposure to the city sound (Figure 6b, p < 0.01). Thus, the forest sound evoked a more relaxed feeling than the city sound. Further, for the natural–artificial scale, the mean score was "moderately natural" with exposure to the forest sound but was "slightly-to-moderately artificial" with exposure to the city sound (Figure 6c, p < 0.01). Thus, the forest sound evoked a more natural feeling than the city sound. Further, for the natural–artificial scale, the mean score was "moderately natural" with exposure to the forest sound but was "slightly-to-moderately artificial" with exposure to the city sound (Figure 6c, p < 0.01). Thus, the forest sound evoked a more natural feeling than the city sound (Figure 6c, p < 0.01).

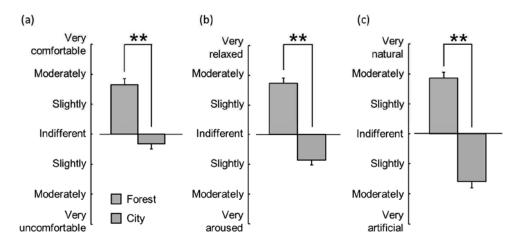


Figure 6. Subjective feelings on three scales (comfortable–uncomfortable (**a**), relaxed–aroused (**b**), and natural–artificial (**c**)), as measured by a modified semantic differential method, after exposure to the forest vs. city sounds. Data are expressed as mean \pm standard error (n = 29). ** *p* < 0.01 determined by the Wilcoxon signed-rank test (one sided).

3.2.2. Profile of Mood States (POMS)

Figure 7 summarizes the results of the POMS questionnaire, showing the mean scores for the six subscales and total mood disturbance score after exposure to the forest or city sounds. Scores for the negative subscales were significantly lower after exposure to the forest sound than those after exposure to the city sound (tension–anxiety (T-A, p < 0.01); depression–dejection (D, p < 0.05); anger–hostility (A-H, p < 0.01); fatigue (F, p < 0.01); and confusion (C, p < 0.01)), whereas the positive mood state of vigor (V, p < 0.01) was significantly higher. The total mood disturbance (TMD) score was significantly lower after exposure to the forest sound than that after exposure to the city sound (p < 0.01).

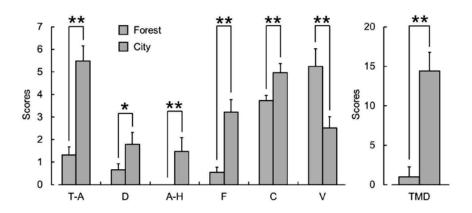


Figure 7. Subjective evaluation of mood states as measured by the six subscales and total mood disturbance score on the profile of mood states (POMS) questionnaire after exposure to the forest vs. city sounds. T-A, tension–anxiety; D, depression-dejection; A-H, anger–hostility; F, fatigue; C, confusion; V, vigor; and TMD, total mood disturbance. Data are expressed as mean ± standard error (n = 29). ** p < 0.01, * p < 0.05 as determined by the Wilcoxon signed-rank test (one sided).

4. Discussion

This study examined the physiological effects of exposure to forest sound on brain and autonomic nervous activities by measuring oxy-Hb concentration in the prefrontal cortex and HRV. Psychological effects were examined through subjective assessments using the modified SD method and POMS questionnaire. Compared with exposure to the city sound, exposure to the forest sound resulted in decreased oxy-Hb concentrations in the right prefrontal cortex and suppression of sympathetic nervous activity and heart rate. Psychologically, exposure to the forest sound resulted in greater feelings of comfort, relaxation, and naturalness, as well as improved mood states, than exposure to the city sound. Taken together, these physiological and psychological responses indicate that nature-derived auditory stimulation induced relaxation effects.

The finding of decreased right prefrontal cortex activity with the exposure to the forest sound was consistent with the results of previous studies on the physiological responses to visual, olfactory, and tactile stimuli [19,26,34]. These studies reported decreased oxy-Hb concentrations in the right prefrontal cortex when viewing a forest image (vs. a city scene) [19], viewing fresh roses (vs. no stimulus) [26], and smelling rose and orange essential oils (vs. odorless air) [34]. The present study's findings provide further confirmation about the calming effect of exposure to a nature-derived auditory stimulation on the prefrontal cortex activity.

The HRV results showed a significant decrease in sympathetic nervous activity during exposure to the forest sound. This was consistent with the results of previous studies that investigated the physiological visual effects of exposure to nature [21,23,24,56,57]. These studies reported decreases in sympathetic nervous activity when viewing real pansies (vs. artificial pansies) [56], bonsai (vs. no stimulus) [21,23], foliage plants (vs. no stimulus) [24], and a three-dimensional image of a water lily (vs. a two-dimensional image) [57]. However, the present study found no significant difference in

terms of parasympathetic nervous activity during exposure to the forest and city sounds. In contrast, some previous studies have shown significant enhancement in parasympathetic nervous activity with nature-derived stimuli [21,25,32,33,35–37]. The reason for the discrepancy between the sympathetic and parasympathetic nervous activities in the present study remains unknown, and this issue requires further study. The present study also found a decrease in heart rate with exposure to the forest sound compared with that to the city sound. This is consistent with the findings of previous studies on the physiological effects of nature-derived stimuli [32,37]. A similar finding was reported in a recent clinical study on patients hospitalized in a cardiac care unit; in the patient group that listened to natural sounds for 30 min, there were significant decreases in heart rate before and after the intervention [58].

A log-transformed LF to HF ratio (ln [LF/HF]) significantly decreased during exposure to forest sound. In this study, LF/HF was considered as an indicator of the relative sympathetic nervous activity. Hence, the present results imply that exposure to forest sound decreases the relative sympathetic nervous activity. This supposition is supported by a significantly lower mean HR during exposure to forest sound. However, this issue remains controversial among researchers. In recent years, some researchers have suggested that the LF of HRV is the result of modulation by baroreflex rather than an index of sympathetic activity [59,60]. On the other hand, this idea has been refuted by other researchers [61]. Considering that this study is not about the physiological interpretation of HRV, we did not specifically discuss this point. The uncertainty of the physiological interpretation of LF/HF is one of the limitations of this study.

The study focused on shorter HR recordings. For short-term HRV measurement, 5 min recording is generally recommended [44]. To investigate acute physiological responses to sound stimulation, we analyzed the HR recording every 60 s. The HRV indicator became unstable due to the short HR recording. The instability of HRV indices in this study increased the probability of Type II errors (false negatives) in statistical tests, but did not increase the probability of Type I errors (false positives). Therefore, the short duration of the HRV measurements did not impair the reliability of the present results that demonstrated a significant decrease in ln(LF/HF) during exposure to forest sounds.

The results of the psychological assessments using the modified SD method showed that exposure to the forest sound increased psychological relaxation, eliciting greater feelings of comfort, relaxation, and naturalness compared with exposure to the city sound. These results are similar to those of previous studies that have compared the effects of forest and city stimuli in field and laboratory experiments [8–16,19]. The POMS questionnaire scores of mood states showed that forest sounds can relieve psychological tension, depression, anger, fatigue, and confusion compared with city sounds, and that they could enhance psychological vigor. Previous studies that investigated the psychological effects of exposure to nature-derived stimuli via other senses have reported similar improvements in mood states, such as in reducing anxiety levels in surgical patients [63] or facilitating a positive change in calm–anxious, energetic–tired, and agreeable–hostile mood states of adults with neurological impairments [64]. The present study demonstrated that the forest sound provided psychological benefits of positive feelings and mood state improvement similar to the effects of music therapy.

In summary, this study showed both physiological and psychological relaxation associated with exposure to the forest sound compared with exposure to the city sound. However, the present study has certain limitations that should be considered. First, we examined the effects of nature-derived auditory stimulation only in women in their 20s. To generalize these effects, further studies are needed with other demographic groups, including individuals of different ages and men. Second, although various natural sounds, such as the sound of the wind, a brook, murmuring trees, or singing birds [65], are associated with forests, we used the sound of a brook as the auditory stimulus. Further studies that use other kinds of natural sounds are needed.

5. Conclusions

The findings of the present study indicated that, when compared with city sounds, exposure to the forest sound induced physiological and psychological relaxation, with decreased oxy-Hb concentrations in the right prefrontal cortex activity, decreased sympathetic nervous activity, lower heart rate, and increased feelings of comfort, relaxation, and naturalness, as well as improved mood states. The study clarified that a nature-derived auditory stimulation induced physiological and psychological aspects of relaxation compared with city-derived auditory stimulation.

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Original article

Physiological effects of forest-related visual, olfactory, and combined stimuli on humans: An additive combined effect



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ABSTRACT

Handling Editor: Matilda van den Bosch Keywords: Forest therapy Heart rate variability Near-infrared spectroscopy Physiological relaxation Preventive medicine Shinrin-yoku The aim of this study was to investigate the physiological effects on brain activity and autonomic nervous activity of forest-related visual, olfactory, and combined visual and olfactory stimuli. Twenty-one female Japanese university students (age, 21.1 ± 1.0 years) participated. In a soundproofed chamber with an artificial climate, each participant was presented for 90 s with the following conditions: an image of a forest landscape of Hinoki cypress trees with no odor (visual stimulus), a grav image with Hinoki cypress leaf essential oil (olfactory stimulus), an image of a forest landscape of Hinoki cypress trees with Hinoki cypress leaf essential oil (combined stimulus), and a gray image with no odor (control). As an indicator of brain activity, oxyhemoglobin concentrations were measured in the left and right prefrontal cortices using near-infrared time-resolved spectroscopy. Heart rate variability and heart rate were used as indicators of autonomic nervous activity. The highfrequency component of heart rate variability, which reflects parasympathetic nervous activity, and the lowfrequency/high-frequency ratio, which reflects sympathetic nervous activity, were evaluated. The following results were obtained in comparison with the control conditions: (1) the combined stimuli resulted in significantly decreased oxyhemoglobin concentrations in the left and right prefrontal cortices; (2) the olfactory stimulus resulted in significantly decreased oxyhemoglobin concentration in the right prefrontal cortex; and (3) the visual stimulus resulted in significantly decreased sympathetic nervous activity related to arousal or situations of stress. Results of a questionnaire indicated that these forest-related stimuli significantly increased the participants' feelings of "comfortable" and "relaxed," with the visual and combined stimuli significantly increasing feelings related to the terms "natural" and "realistic." In conclusion, forest-related visual, olfactory, and combined visual and olfactory stimuli induced physiological and psychological relaxation effects, and the combined visual and olfactory stimuli exhibited an additive effect.

1. Introduction

In modern urbanized societies, people are constantly overexposed to stressors, increasing their risk of health problems (Tanaka et al., 1996; Peen et al., 2010; Lederbogen et al., 2011; McKenzie et al., 2013). Thus, there is a need for effective methods for coping with stress and inducing relaxation. One such method that has received focus is interaction with forests, with recent studies reporting positive effects on physical and mental health (Song et al., 2016; Hansen et al., 2017; Miyazaki, 2018). Investigating the relaxation effect associated with forest interactions and elucidating the underlying mechanisms may therefore be of considerable potential benefit.

Many studies have demonstrated that spending time in forests, such

as walking in a forest or viewing a forest landscape, can mitigate stress states (Park et al., 2007; Tsunetsugu et al., 2007; Park et al., 2008; Lee et al., 2009; Park et al., 2009, 2010; Lee et al., 2011; Tsunetsugu et al., 2013; Lee et al., 2014; Song et al., 2019) and that participation in a forest therapy program can induce physiological relaxation (Ochiai et al., 2015a; Ohe et al., 2017; Song et al., 2017a; Bielinis et al., 2019). Experiments with elderly people and with adults at risk of stress- and lifestyle-related diseases, such as high blood pressure, diabetes, and depression, have shown positive physiological effects with various activities performed in forests (Ohtsuka et al., 1998; Mao et al., 2012; Sung et al., 2012; Lee and Lee, 2014; Ochiai et al., 2015b; Song et al., 2015; Chun et al., 2017; Song et al., 2017b). These investigations of human physiological responses at field sites have been valuable,

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Abbreviations: Oxy-Hb, oxyhemoglobin; HRV, heart rate variability; HF, high frequency; LF, low frequency; TRS, near-infrared time-resolved spectroscopy * Corresponding author.

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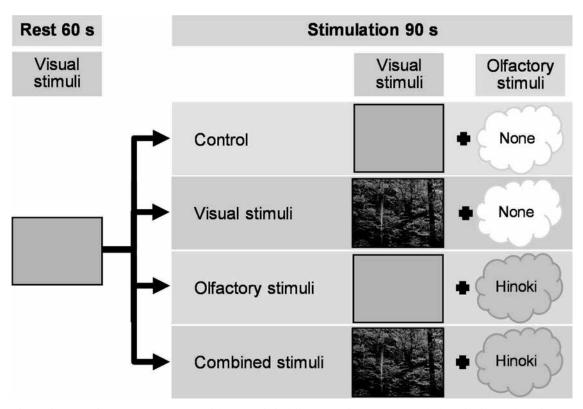


Fig. 1. The stimulus conditions. Each participant was presented for 90 s with the following conditions: an image of a forest landscape of Hinoki cypress trees with no odor (visual stimulus), a gray image with Hinoki cypress leaf essential oil (olfactory stimulus), an image of a forest landscape of Hinoki cypress trees with Hinoki cypress leaf essential oil (combined stimulus), and a gray image with no odor (control). The order of the stimulus conditions was varied to avoid order bias.

revealing the overall effects of the surrounding environment (Lee et al., 2012).

However, clarifying the physiological effects of forests on humans requires laboratory studies in addition to those in the field (Lee et al., 2012). A field study is valuable for revealing the effects of forest interactions, but the ever-changing conditions mean that reproducibility cannot be assured. Conversely, laboratory studies can produce reproducible results and can test the effects induced by specific types of stimuli. Another advantage of laboratory studies is that they allow more detailed measurements, thereby providing greater insight into the physiological mechanisms that lead to a relaxed state. However, compared with field experiments, there have been few laboratory experiments on the impact of forest interactions on humans.

Forest-based stimuli are subconsciously perceived through the five senses. Of the five senses, physiological effects of olfactory stimulation by volatile phytoncides derived from trees have been characterized in detail. In addition, laboratory studies using essential oils extracted from trees such as Taiwan cypress and Hinoki cypress have demonstrated that inhalation of these forest-related smells induced a decrease in blood pressure (Miyazaki et al., 1992), an increase in parasympathetic nervous activity, which is known to be associated with a relaxed state (Ikei et al., 2015), a decrease in oxyhemoglobin (oxy-Hb) concentration in the prefrontal cortex (Ikei et al., 2015), and an increase in natural killer cell activity and improvement of immune functions (Li et al., 2009). In addition, it has been reported that inhalation of α -pinene and D-limonene, which are major components of forest odors, resulted in decreased blood pressure (Tsunetsugu et al., 2012), enhanced parasympathetic nervous activity, and decreased heart rate (Joung et al., 2014; Ikei et al., 2016).

In contrast to the extensive study of olfactory stimuli, investigations of forest-related visual, auditory, and tactile stimuli have been limited. However, there have been several studies related to visual stimuli. Song et al. (2018) investigated brain and autonomic nervous activity, measured using indices of prefrontal cortex activity, heart rate variability, and heart rate, while participants viewed an image of either a forest or a city; this demonstrated that visual stimulation with the forest image induced a decrease in oxy-Hb concentration in the prefrontal cortex. Ulrich et al. (1991) investigated the effects of viewing slides of forests or cities on stress states, measured using electroencephalography indices and heart rate. Stress states rapidly decreased when viewing slides of a forest compared with viewing slides of a city.

Although there have been studies confirming the effects of either visual or olfactory stimuli alone, there has been little investigation of the effect of both types of stimuli presented in combination. The aim of this study, therefore, was to investigate the physiological effects induced by forest-related visual, olfactory, and combined visual and olfactory stimuli. The physiological responses measured were brain activity [oxy-Hb concentration in the left and right prefrontal cortices, assessed with near-infrared time-resolved spectroscopy (TRS)] and autonomic nervous activity (sympathetic and parasympathetic nervous activity, evaluated from heart rate variability (HRV), and heart rate).

2. Materials and methods

2.1. Participants

The participants were 21 female Japanese university students (mean \pm standard deviation; age, 21.1 ± 1.0 years; height, 158.2 ± 4.5 cm; and weight, 51.9 ± 6.2 kg). They were recruited via bulletin board advertising at the university. The study excluded anyone who smoked, who was currently receiving treatment for any disease, or who was in her menstrual period at the time of the study. After being informed about the aims and procedures of the study, the participants gave their written consent to take part. The study was conducted in accordance with the guidelines of the Declaration of Helsinki, and the protocol was approved by the Ethics Committees of the Center for

Environment, Health and Field Sciences, Chiba University, Japan (project identification no. 5).

2.2. Experimental design

The experiments were performed in a soundproofed chamber with an artificial climate in the Center for Environment, Health and Field Sciences, Chiba University. The chamber was maintained at 24 °C, with 50% relative humidity and 50-lux illumination. The participant was provided with a description of the purpose and outline of the study while waiting in a waiting room. She then entered the chamber, where sensors for the physiological measurements were fitted and received a description of the measurement procedure. Throughout the testing procedure, the participant remained sitting, and her physiological responses were continually measured. The participant viewed a gray background for 60 s (the rest period), followed by the visual, olfactory, combined visual and olfactory stimuli and the control for 90 s (Fig. 1).

After finishing the 90-s stimulus period, the subjective indices were evaluated. To eliminate order bias, the order of the stimulus conditions was varied between participants.

2.3. The stimuli

The visual stimulus was a photograph view of a forest landscape of Hinoki cypress trees (*Chamaecyparis obtusa*, a type of conifer) displayed on a 4K-compatible high vision liquid crystal display television of width 1872 mm, height 1053 mm, and pixel resolution 3840×2160 (85 V type, TH-85AX900; Panasonic, Osaka, Japan). The participant sat 1.4 m away from the display (Fig. 2A).

The olfactory stimulus was Hinoki cypress leaf oil (Kiseitec Co., Wakayama, Japan) extracted by steam distillation from leaves and twigs of Hinoki cypress trees growing in Wakayama Prefecture, Japan. The oil (2 μ L) was injected into a 24-L odor bag (polyethylene terephthalate film heat seal bag; NS-KOKEN Co., Ltd., Kyoto, Japan). The odor bag was exposed for approximately 1 h at room temperature to diffuse the essential oil into the bag. The odor was presented to the participant via a device fixed to her chest approximately 15 cm under her nose (Fig. 2B), through which air saturated with the essential oil was pumped at 3 L/min. In preliminary investigations, the subjective intensity of the odor was described as "weak" or "easily sensed."

The combined stimulus was a combination of visual and olfactory stimuli, and the control was set to gray image with an odorless condition to generate no stimulus by the forest.

2.4. Physiological measurements

2.4.1. Near-infrared time-resolved spectroscopy

As an indicator of brain activity, oxy-Hb concentration in the participant's left and right prefrontal cortex was measured during the 60-s rest period and the 90-s stimulus period using TRS (Ohmae et al., 2006, 2007). The sensors of a TRS-20 system (Hamamatsu Photonics K.K., Shizuoka, Japan) were mounted on the participant's forehead at approximately the Fp1 and Fp2 positions of the international 10–20 electroencephalography system.

These measurements are based on the following principles. An increase in local brain activity results in increased blood flow and perfusion, with the blood flow exceeding oxygen consumption (Fox and Raichle, 1986). As a consequence, oxy-Hb increases; TRS detects this increase using near-infrared spectrometry. It has been shown that increases and decreases in blood flow in the brain are consistent with those of oxy-Hb (Hoshi et al., 2001), and a decrease in oxy-Hb concentration is thought to result in physiological calming (Hoshi et al., 2011).

In the present study, measurements were made at approximately 1.0–1.2 s intervals. The data were then transformed using linear interpolation to produce a time series of oxy-Hb concentrations at 1.0-s intervals. The average concentration for the 60-s rest period was calculated and used as the baseline value for the subsequent stimulus period. This baseline value was subtracted from each data point during the stimulus period, and the average value over the 90-s stimulus period was used in the analysis.

2.4.2. Heart rate variability and heart rate

HRV and heart rate were used to provide indicators of autonomic nervous activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Kobayashi et al., 1999). The periods between consecutive R waves (R-R intervals) measured by a portable electrocardiogram (Activtracer AC-301A; GMS, Tokyo, Japan) were analyzed to obtain the heart rate and HRV. The power levels of the low-frequency (LF; 0.04-0.15 Hz) and high-frequency (HF; 0.15-0.40 Hz) components of HRV were calculated using the maximum entropy method using MemCalc/Win software (GMS, Tokyo, Japan) (Kanaya et al., 2003). To normalize the HRV parameters across participants, we used natural logarithmic-transformed values in the analysis (Kobayashi et al., 2012). The HF component of HRV reflects parasympathetic nervous activity and the ratio of LF to HF reflects sympathetic nervous activity (Pagani et al., 1986; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Average values of ln(HF), ln(LF/HF), and heart rate over the 90-s stimulus period were used in the analysis.

The HRV power spectra were also used to estimate the participant's respiratory frequency (Schäfer and Kratky, 2008). Respiratory changes influence HRV data, with heart rate generally increasing during inspiration and decreasing during expiration (McCrady et al., 1966; Kobayashi, 1998); thus, the respiratory rate can be estimated from the dominant frequency of the HF component, found by detecting the maximal power of HF component. To detect the peak frequency of the

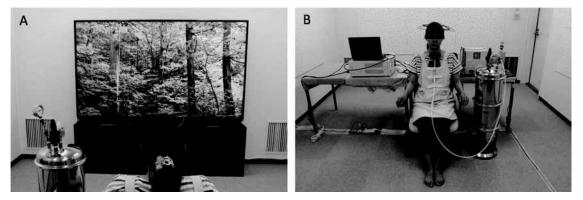


Fig. 2. The experimental set-up. (A) The visual stimulus. (B) Apparatus for presenting the olfactory stimulus.

HF component, the model order for the spectral analysis was chosen from eighth to the thirteenth; however, the ninth order was used in principle.

2.5. Psychological measurements

The modified semantic differential method (Osgood et al., 1957) was used to evaluate the participant's psychological responses to the stimuli. This method tests the participant's subjective spatial impressions through a questionnaire comprising four pairs of opposite adjectives, each evaluated on 13 scales, including "comfortable to uncomfortable," "relaxed to aroused," "natural to artificial," and "realistic to unrealistic."

2.6. Statistical analyses

The HRV data of 17 of the participants were used in the analysis because of errors in data collection for four participants. The software package SPSS v21.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. The participants' physiological responses to the different stimulus conditions (visual stimulus, olfactory stimulus, and combined visual and olfactory stimuli) and the control were compared using paired t tests with the Holm correction (Victor et al., 2010; Eichstaedt et al., 2013). The Holm correction was therefore applied three times, using the following procedure. All the p-values were sorted by size and compared with increasing limits. The lowest limit was the overall significance limit (0.05) divided by three. The smallest P-value was compared with this (0.05/3 \approx 0.017); if it was greater than this value, the process stopped; if it was less than this value, the significance limit was divided by two (0.05/2 = 0.025) and the process repeated. If necessary, the next value for comparison was the significance limit divided by one (i.e., P = 0.050). The Wilcoxon signed-rank test with Holm correction was applied to evaluate the differences in psychological indices between the stimulus conditions and the control. Onesided tests were used for both sets of comparisons

3. Results

3.1. Physiological effects

3.1.1. TRS

Fig. 3 shows the mean time courses of oxy-Hb concentration in the left and right prefrontal cortices during the stimulus period for each of the stimulus conditions. The baseline values of oxy-Hb concentration (i.e., the average values for the 60-s rest period prior to the stimulus period) did not differ significantly among the four conditions in either [mean ± standard prefrontal cortex (left errorl: control. $42.71 \pm 1.27 \,\mu\text{M};$ visual, $42.84 \pm 1.21 \,\mu\text{M};$ olfactory, $42.75 \pm 1.19 \,\mu\text{M}$; and combination, $42.86 \pm 1.15 \,\mu\text{M}$; P > 0.05; right: control, 42.05 \pm 1.03 μ M; visual, 42.43 \pm 1.06 μ M; olfactory, $42.53 \pm 1.00 \,\mu\text{M}$; and combination, $42.74 \pm 1.08 \,\mu\text{M}$; P > 0.05). The oxy-Hb concentrations in the left and right prefrontal cortices decreased after the start of the stimulus. With the control condition, the concentration then gradually increased; with the other three conditions, the concentrations remained lower than baseline throughout the rest of the stimulus period.

Average values of the oxy-Hb time course relative to baseline during each stimulus period were calculated for each participant; the mean values of these averages are shown in Fig. 4. In the left prefrontal cortex, there was a significant difference between the combined stimulus and control conditions (control, $-0.08 \pm 0.10 \,\mu$ M; combination, $-0.48 \pm 0.09 \,\mu$ M; P < 0.05; Fig. 4, left), but no significant differences between the visual ($-0.22 \pm 0.08 \,\mu$ M) or olfactory ($-0.37 \pm 0.11 \,\mu$ M) and control conditions. In the right prefrontal cortex, both the combined stimulus condition and the olfactory condition showed a significant difference from the control condition (control,

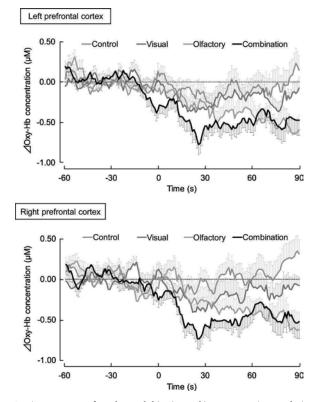


Fig. 3. Time courses of oxyhemoglobin (oxy-Hb) concentrations, relative to baseline, in the left and right prefrontal cortices during the rest periods (-60 to 0 s) and the stimulus periods (1-90 s). Each line corresponds with one of the three forest-related stimulus conditions (visual, olfactory, and combined visual and olfactory) and control. Each data point represents the mean \pm standard error for the 21 participants.

 $0.02 \pm 0.10 \,\mu$ M; olfactory, $-0.32 \pm 0.12 \,\mu$ M; combination, $-0.46 \pm 0.08 \,\mu$ M; P < 0.05; Fig. 4, right). No significant difference was observed for the visual stimulus condition ($-0.19 \pm 0.10 \,\mu$ M).

3.1.2. HRV and heart rate

There was no significant difference among the four conditions in mean respiratory frequency (control, 0.28 ± 0.01 Hz; visual, 0.27 ± 0.01 Hz; olfactory, 0.25 ± 0.02 Hz; combination, 0.25 ± 0.02 Hz; P > 0.05).

Fig. 5 shows the overall mean values for ln(HF) and ln(LF/HF) derived from HRV during the four stimulus conditions. A trend similar to the results of prefrontal cortex activity was observed for ln(HF), which is an index of parasympathetic nervous activity, although the differences were not statistically significant (control, 6.32 ± 0.21 lnms²; visual, 6.37 ± 0.23 lnms²; olfactory, 6.46 ± 0.23 lnms²; combination, 6.51 ± 0.27 lnms²; P > 0.05; Fig. 5, left). There was a significant difference among the stimulus conditions for ln(LF/HF), which is an index of sympathetic nervous activity: the value for the visual stimulus condition was significantly lower than that for the control condition (control, -0.26 ± 0.17 ; visual, -0.67 ± 0.17 ; P < 0.05; Fig. 5, right). However, no statistically significant differences were found between the olfactory (-0.45 ± 0.21) or the combined (-0.41 ± 0.29) stimulus and the control conditions.

There was no significant difference among the four conditions in heart rate (control, 69.7 \pm 1.8 bpm; visual, 69.8 \pm 1.9 bpm; olfactory, 70.4 \pm 2.0 bpm; combination, 70.3 \pm 2.0 bpm; P > 0.05, data not shown).

3.2. Psychological effects

Fig. 6 summarizes the results of the modified semantic differential

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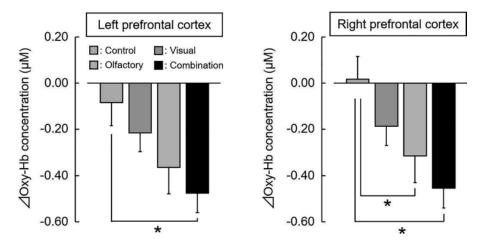


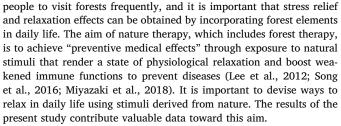
Fig. 4. Mean values of the participants' average oxyhemoglobin (oxy-Hb) concentration in the left and right prefrontal cortices during the stimulus periods for the three forest-related stimulus conditions (visual, olfactory, and combined visual and olfactory) and control. The data are shown as mean \pm standard error; n = 21. * P < 0.05, as determined by a paired *t*-test (one-sided) with the Holm correction.

method analysis of the participants' subjective feelings after exposure to the four stimulus conditions. The participants felt more "comfortable" and more "relaxed" following exposure to the three forest-related stimulus conditions compared with exposure to the control condition (P < 0.05). In addition, the visual and combined stimulus conditions evoked increased feelings related to the terms "natural" and "realistic" compared with the control condition (P < 0.05).

4. Discussion

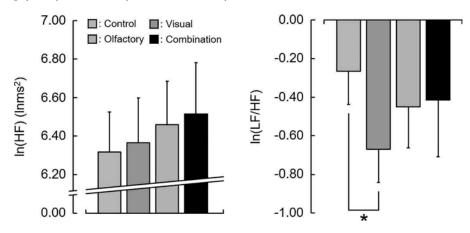
In this study, forest-related visual, olfactory, and combined visual and olfactory stimuli evoked physiological and psychological relaxation effects. The following findings were obtained in comparison with the control condition. (1) The combined visual and olfactory stimuli resulted in significantly decreased oxy-Hb concentrations in the left and right prefrontal cortices. (2) The olfactory stimulus resulted in significantly decreased oxy-Hb concentration in the right prefrontal cortex. (3) The visual stimulus resulted in significantly decreased sympathetic nervous activity. In addition, the questionnaire results showed that these forest-related stimuli significantly increased "comfortable" and "relaxed" feelings and the visual and combined stimuli significantly increased feelings related to the terms "natural" and "realistic." These findings are consistent to some extent with those of previous studies that examined physiological and psychological responses to a forest-related stimuli (Park et al., 2007; Tsunetsugu et al., 2007; Park et al., 2009, 2010; Lee et al., 2011; Tsunetsugu et al., 2013; Lee et al., 2014; Ikei et al., 2015; Song et al., 2018, 2019).

These findings support the possibility that simply exposure to a familiar forest-related stimulus for a short period, such as viewing forest images or smelling tree-derived essential oils, can be relaxing both physically and mentally. In modern society, it is difficult for most



A further finding of the present study was that different types of forest-related stimuli evoked different physiological responses. There have been few previous studies on single or combined stimuli such as those used in this study, so the underlying mechanism is unknown. However, there was a tendency for the participants to show greater relaxation in response to the combined stimuli than to each single stimulus, demonstrated by prefrontal cortex activity, parasympathetic nervous activity, and the subjective feelings related to the terms "comfortable," "relaxed," "natural," and "realistic." There appears to have been an additive effect. These results suggested that when there is a large amount of stimulus information, the physiological and psychological relaxation effects increase. However, it is unclear why a similar tendency was not observed with sympathetic nervous activity. For more specific consideration of this, future studies should accumulate and examine data using various forest-related stimuli. Furthermore, olfactory stimuli exhibited a significant difference in the right prefrontal cortex only. Olfactory stimuli, such as rose and orange essential oils (Igarashi et al., 2014) and Hinoki cypress leaf oil (Ikei et al., 2015), can result in physiological relaxation, as revealed by oxy-Hb concentration variations in the right prefrontal cortex, without exhibiting significant changes in the left prefrontal cortex. These results are in agreement with our findings; however, the mechanism remains unknown. Hence,

> **Fig. 5.** Summary of the heart rate variability analyses. The graphs show overall mean values for natural logarithmic-transformed high-frequency (HF) and low-frequency to high-frequency ratio (LF/HF) values for the three forest-related stimulus conditions (visual, olfactory, and combined visual and olfactory) and control. The data are expressed as mean \pm standard error, n = 17. * P < 0.05 as determined by a paired *t*-test (one-sided) with the Holm correction.



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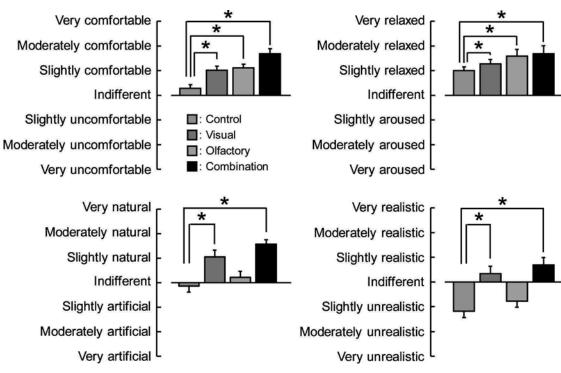


Fig. 6. The participants' subjective feelings, as measured by a modified semantic differential method, after exposure to the three forest-related stimulus conditions (visual, olfactory, and combined visual and olfactory) and control. The data are expressed as mean \pm standard error (n = 21). * P < 0.05, as determined by the Wilcoxon signed-rank test (one-sided) with the Holm correction.

the differences in the activation of the left and right prefrontal cortices need to be verified.

Conversely, the questionnaire results indicated that the visual and combined stimuli significantly increased the participants' feelings related to the terms "natural" and "realistic." This suggested that appropriate visual stimuli may increase people's feelings that something is "natural" and "realistic." Thus, to enhance the subjective feeling of "natural" when, for example, designing an urban space, it is important to use visual stimuli well.

The findings of this study provide objective evidence for the beneficial physiological and psychological effects of forest-related visual, olfactory, and combined visual and olfactory stimuli for young women in their 20 s. However, generalization of these findings would require further studies based on larger samples that include other demographic groups, such as men and different age groups. It is also necessary to examine such effects in populations who experience a heightened state of stress in daily life. In addition, the present study used a stimulus derived from Hinoki cypress forest during summer. The effects of various forest-derived stimuli in different seasons need to be elucidated in future studies.

5. Conclusions

This study examined the effects of forest-related visual, olfactory, and combined visual and olfactory stimuli on brain and autonomic nervous activity. The stimuli induced a significant decrease in the oxy-Hb concentration in the prefrontal cortex, a significant decrease in sympathetic nervous activity, and significant increases in subjective feelings related to the terms "comfortable," "relaxed," "natural," and "realistic." In conclusion, the forest-related stimuli promoted physiological and psychological relaxation effects, and the combined visual and olfactory stimuli demonstrated an additive effect.

CRediT authorship contribution statement

Chorong Song: Conceptualization, Data curation, Formal analysis,

Investigation, Methodology, Visualization, Writing - original draft. Harumi Ikei: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization. Yoshifumi Miyazaki: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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おわりに

森林は大気・淡水循環に伴い、CO₂の炭素固定や、気候緩和、砂漠化防止、生物多様 性維持等、地球環境保全のために重要な生態系ですが、1982 年、当時の林野庁長官に よる「森林浴」とその医・科学的検証の提唱以来、日本では都市部に居る時との比較で、 森林内では、ストレスが緩和され、リラックス度が増し、免疫機能の一つである NK 細 胞の数・活性や抗癌蛋白質の活性が増す等、ヒトの生理的反応が、健康維持・増進、疾 病予防に寄与すると、証明され、中でもストレスとリラックスについては、唾液・尿・ 血液中のストレスホルモン測定、自律神経活動や脳活動の測定等々、その検体や測定方 法を異にしても同様の結果が得られました。

以上は論文、著書、報道、取材、学会発表等を経て、森林の重要性の新分野として、 国際的に認められ、「Shinrin-yoku」も国際用語となり、その広がりは、研究分野にお いては、先進諸国でも注目されていますが、今般の事業担当者宮崎先生に師事し、帰国 後研究を継続した研究者たちや、本事業に携わり先頃帰国した研究者を含む韓国はじめ マレーシア等が共同研究も視野に入る一方で、北欧、英、米、中国、オセアニア、スリ ランカ、その他 Shinrin-yoku の実践を計る国々も増えています。

さて、本事業の第Ⅰ期、第Ⅱ期における実証実験を含む研究の持つ意義ですが、それ までの研究の多くは若齢の健常な男女を対象とした実験でした。今般の対象者は、高血 圧者を含む中高齢男女や、日頃ストレスフルであろう脊髄損傷車椅子使用者、外来通院 のうつ病患者で行われました。後二者については、屋内外での実験のみでしたが、スト レスは、生活習慣病や精神性疾患を含む多くの疾患の誘因及び悪化の要因となるため、 有疾患者のストレス低減を明らかとした結果は、疾病増悪の予防に、有意義であったと 示唆され、今後、他の疾患に対する実証実験への出発点となります。又、地球環境に必 須な森林の維持に欠かせない生態系サービスビジネスにも役立つと考えら、この実験結 果は、日本発で、研究、実践共に世界的に拡大する可能性も大です。

又、「森林浴」提唱時、その歴史的経緯から森林浴効果は、嗅覚刺激によるとの概念 が長年ありました。従って、視覚、聴覚、嗅覚単独及び視覚と嗅覚、視覚と聴覚の複合 刺激実験は、概念を覆すものであり、特に視覚と嗅覚複合刺激では、リラックス度に両 者の加算効果が表れており、Shinrin-yoku 中は、五感を集中的に駆使する事がストレ スを緩和しリラックスする手法の一つと示唆されるため、この判明は、今後の Shinrinyoku の実践に多大な一助を成したと言えます。この判明も又、世界的に初めての発見 です。

今後は以上の実験成果を踏まえ、さらに世界をリードする新たな視点、手法による、 国民の健康増進を目的とした調査研究事業の継続に期待します。

令和元年11月

森林浴による健康増進等に関する調査研究委員会

委員長今井通子

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