近年身の周りの化学物質の増加とそれに伴う種々の健康影響が懸念され,特にシックビルディング症候群・シックハウス症候群や化学物質過敏症といった問題が大きな社会問題となって来ている [10−12]. ホルムアルデヒドがこれらの主要原因物質の一つとされ,改めてホルムアルデヒドの生体影響が注目されて来ている. これらのホルムアルデヒドを取り巻く最近の動向については前報[13]に示したように,各種行政対応も取られて来ている. すなわち旧厚生省は,平成9年6月ホルムアルデヒドの室内濃度指針値について検討し, WHOのガイドライン値80 ppb(0.1 mg/m^3)を室内濃度指針値として提案した [14]. さらに平成13年4月には文部科学省高等教育局医学教育課長名により「医学生及び歯学生の系統解剖実習時の環境向上について」という通知が出され,全国的に調査が進められている [15]. また平成14年厚生労働省より「職域における屋内空気中のホルムアルデヒド濃度低減のためのガイドライン」が示された [16].

本報では,解剖学実習における学生および教職員の安全性評価のために,解剖学実習の環境改善および防備体制などの充実に繋げる基礎データを蓄積することを目的として,解剖実習室の環境中濃度測定を行うと同時に自覚症状調査を試みた.

対象と方法

1. 気中ホルムアルデヒド測定

1-1. 実習室空気のサンプリング

本学の医学部解剖学実習室(19.5 m×24 m×3 m, 1400 m^3)は,地上8階,地下1階の建物の地下1階に位置している. 実習室において,実習が開始される前の週,および実習期間中3回に渡って室内空気の捕集を行った. サンプリングは平成13年,14年の2年に渡って実施した. 作業環境測定基準に準じて,縦横それぞれ等間隔の交点を測定点とした. 解剖室内のA測定点は全部で12点,サンプリング口の高さは1.5 mとした. B測定点は解剖台の周りで実習している学生の位置で,頭位の高さに調整し2もしくは3点とした. 解剖実習室におけるサンプリングデザインをFig. 1に示す. 気中ホルムアルデヒドは,ミニポンプMP-Σ30(柴田科学,東京)を用いて,流量0.5 ℓ/minでA測定点は30分間,B測定点は10分間,空気を2, 4-dinitrophenylhydrazine(DNPH)含浸カラムSep-Pak XpoSure Aldehyde Sampler(Nihon Waters K.K., Tokyo)に吸引捕集した. なお,1班4人で一体の献体を解剖し,24班が同時に実習を行っていた. 解剖実習時の解剖室内の窓は閉じられており,換気は中央管理により100%外気を給気し再循環を行わない換気システムにより空調が行われていた. 給気は天井に均等に配置された12箇所の給気口からなされ,排気は教卓後ろおよび前方側壁に設けられた排気口から行われていた(Fig. 1).

また,実習室内に吸着剤としてピュラフィル(ニッタ(株),大阪)を装備した空気清浄装置・アルサス特殊型(東洋空気調和株式会社,東京)が11台設置されているが,その効果を検討するために装置の吸入口と排気口において同様のサンプリングを実施した. さらに,解剖実習室から周辺環境へのホルムアルデヒド含有空気の漏洩の有無を確認するために,実習室に通じる廊下,およびエレベーターホールにて同様のサンプリングを実施した. 廊下は全長30 mあり,平成13年度は4点を,平成14年度は5点を均等区分して測定した.

対照として,実習室がある建物とは別棟になっている産業保健学部の室内空気および大学構内の室外空気をサンプリングし試料とした.

1-2. ホルムアルデヒドの定量分析

カラムに捕集されたアルデヒド類はアセトニトリルで溶出し,逆相型カラムを用いる高速液体クロマトグラフ(HPLC)にて分離・定

量した.すなわち,装置はSHIMADZU LIQUID CHROMATOGRAPH LC-10(島津製作所,京都),カラムはWakosil-(II)5C18(250 mm×0.4 mm, I.D.)(和光純薬,大阪),移動相は0.2 M酢酸/アセトニトリル=35/65(v/v),測定波長は360 nmで行った[16, 17].ホルムアルデヒド-2,4-DNPHの典型的なクロマトグラムおよび検量線をFig. 2 aおよびbに示す.非常に幅広い濃度域に渡って,直線性を有しており,定量測定が可能である.

2.自覚症状アンケート調査

実習学生など25名に自覚症状についてアンケートを実施した.Fig. 3に示す項目について気にならない場合を「0」とし,不快な場合はマイナス1～3に,快適な場合はプラス1～3に,それぞれ3区分,合計で7区分に得点化し,実習室内と普段の生活における自覚症状について,paired *t*-testにより比較検討した.

結　　果

1.気中ホルムアルデヒド濃度測定

平成13年度測定結果をTable 1に,平成14年度測定結果をTable 2に示した.

一般の労働環境で作業環境測定を実施した場合には,測定ポイント間の差違が大きく標準偏差が非常に大きくなることが多い.そのために作業環境測定では幾何平均で評価される.今回の測定結果では解剖実習室内のホルムアルデヒド濃度はほぼ均一状態を示してお

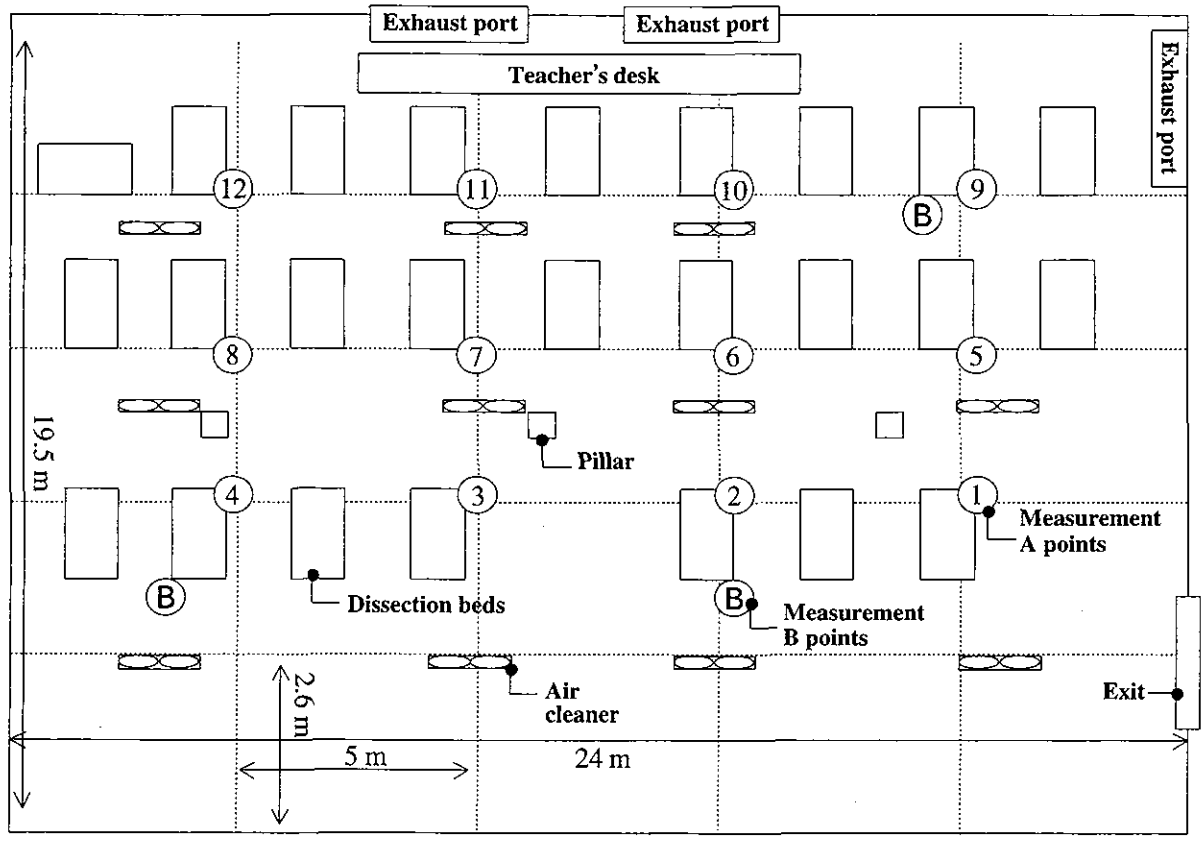

Fig. 1. Design for air sampling in the gross anatomy laboratory.

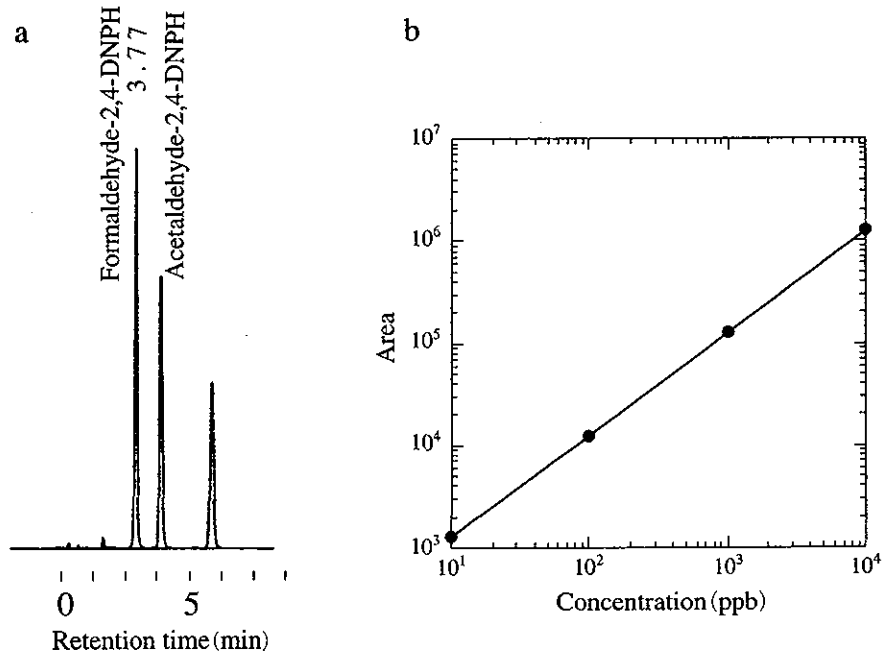

Fig. 2. a: typical chromatogram for formaldehyde-2, 4-DNPH detected by HPLC, b: calibration line.

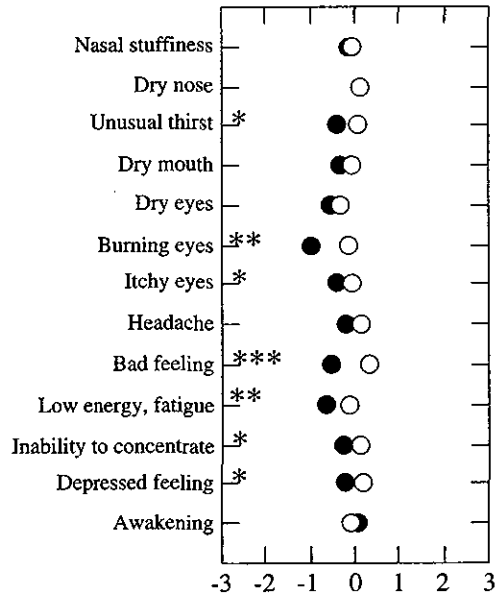

Fig. 3. Comparison of the symptoms of students between the period of exposure to formaldehyde in the gross anatomy laboratory (●), non-exposure period (○). (*$P<0.05$, **$P<0.01$, ***$P<0.001$, paired t-test).

Table 1. Summary of formaldehyde concentration (ppb) at an anatomy laboratory in 2001

Place		No.	1st time Oct/04/2001 (Thu)	2nd time Oct/05/2001 (Fri)	3rd time Nov/12/2001 (Mon)	4th time Dec/14/2001 (Fri)
Anatomy laboratory	Measurement A (Environmental measurement)	1	95	501	1261	1266
		2	104	552	1158	872
		3	116	443	1273	813
		4	75	621	1378	1173
		5	91	559	1391	912
		6	92	747	1303	859
		7	108	476	1491	1078
		8	99	691	1401	1020
		9	82	727	1412	722
		10	85	702	1329	1125
		11	72	568	1653	1084
		12	111	668	1580	910
	Arithmetic mean		94	605	1386	986
	SD		14	103	138	163
	Geometric mean		93	596	1380	974
	SD		1.2	1.2	1.1	1.2
	Measurement B (Personal measurement)	1	–	883	**2060**	1087
		2	–	883	1409	**1709**
		3	–	**914**	1574	
Passage leading to anatomy laboratory		1	–	–	794	–
		2	–	–	318	–
		3	–	–	88	–
		4	–	–	5	–
Indoor (6607)		1	–	–	12	–
		2	–	–	9	–
		3	–	–	14	–
		4	–	–	16	–
		Mean	–	–	13	–
Outdoor		1	–	–	10	–
		2	–	–	10	–
		Mean	–	–	10	–

"indoor (6607)" shows formaldehyde concentration sampled in a general lecture room at a different building as an indoor control. Two or three points were prepared for personal measurement. The value for measurement B was defined as the maximum value shown in bold. SD, standard deviation.

り，幾何平均値も算術平均値もほとんど同じであった．Table 1の平成13年度の測定において，解剖実習開始前日のホルムアルデヒドの測定結果は幾何平均値93 ppb，解剖学実習初日（10月 5 日）の解剖実施中のホルムアルデヒド測定結果は幾何平均値596 ppb，実習期間中盤の開胸，開腹され飛散面積が大きいと思われる時期（11月12日）においては幾何平均値で1380 ppb と高濃度を認めた．また B 測定として最も高濃度曝露される作業者（実習生）の口の高さにおいては2060 ppb の高値を示した．

平成14年度実習に際しては，平成13年度の測定実績を考慮して，ホルマリン固定液の飛散を防ぐように種々の工夫を重ねた．具体的には，解剖台に溜まっている固定液を頻繁に除去する，解剖・観察を終えた臓器を適切に解

Table 2. Summary of formaldehyde concentration (ppb) at an anatomy laboratory in 2002

Place		No.	1st time Oct/04/2002 (Fri)	2nd time Oct/10/2002 (Thu)	3rd time Nov/07/2002 (Thu)	4th time Dec/06/2002 (Fri)
Anatomy laboratory	*Measurement* A (Environmental measurement)	1	31	467	432	699
		2	28	675	624	955
		3	29	799	875	880
		4	16	708	707	1177
		5	31	628	591	1120
		6	15	512	949	1030
		7	16	1049	1006	900
		8	14	577	968	1071
		9	16	732	599	1151
		10	33	529	1150	1314
		11	16	731	838	944
		12	15	625	805	1045
		Arithmetic mean	22	669	795	1024
		SD	8	156	209	162
		Geometric mean	20	654	768	1011
		SD	1.4	1.2	1.3	1.2
	Measurement B (Personal measurement)	1	–	638	**1333**	**1364**
		2	–	**805**	582	779
		3	–	–	1262	1060
Passage leading to anatomy laboratory		1	49	135	40	56
		2	74	199	38	51
		3	64	94	14	35
		4	31	90	–	–
		5	17	21	–	–
Elevator hall		2F	36	9	8	39
		4F	37	9	–	35
		6F	32	9	13	42
		8F	33	10	9	39

Two or three points were prepared for personal measurement. The value for measurement B was defined as the maximum value shown in bold. SD, standard deviation.

剖台から取り除き密閉容器に移すなどの対応がとられた. 結果は Table 2に示すように, 実習開始直後(10月10日)の濃度は前年とほぼ同様幾何平均値654 ppb であった. 実習期間中の中盤(12月 6 日)においては飛散面積の増加につれ前年同様に幾何平均値で1012 ppb と高濃度を認めた. またB 測定値は1364 ppb の高値を示した.

なお, 平成14年度は換気流量から実習室の換気回数を評価した. 実習室換気設定回数は大学開設当初は 1 時間当り換気流量14,800 m^3, 換気回数で10.6回に設定されていた. ところが, 設備の老朽化などに伴い, 平成14年度実習開始時はそれぞれ, 8,000 m^3, 5.7回と低値で換気が十分でなかった. その後, 対策の一つとして排気装置の活性炭フィルター交換, 全熱交換器点検整備が平成14年12月までに実施された. その結果, 1 時間当り換気流量12,060 m^3, 換気回数8.6回まで回復した. 平成14年度12月 6 日の測定値は, この整備直後の値である. この値は, 平成13年度ピークを認めた11月12日の実習中盤の測定値1380 ppb よりは, 若干低下しているが, 平成14年度の換気設備の改修前の測定値768 ppb よりは増加していた.

Table 3. Effects of air cleaners on formaldehyde concentration

Apparatus number	Inhale (ppb)	Exhale (ppb)	Exhale/Inhale
1	408	190	0.47
2	484	304	0.63
3	508	297	0.59
4	649	381	0.59

2. 周辺環境への影響

解剖実習室から周辺環境へホルムアルデヒドを含む空気の漏洩があるか評価するために，実習室に通じる廊下の測定も実施した．Table 1に平成13年度測定中，最も高値を示した11月12日の測定と同時に廊下などを調査した結果を示す．実習室を出て直後の廊下（測定点1）では794 ppbと高値を示したが，実習室から離れると次第に低下し，地下1階のエレベーター周辺（測定点4）では5 ppbと一般環境と相違なかった．上記の測定と同時に室内環境の対照測定とした産業保健学部の一般室（6607講義室）の平均値は13 ppb，また屋外キャンパスの空気中濃度は10 ppbであった．同様に平成14年度も解剖室に通じる廊下およびエレベーターホールを調査した結果をTable 2に示す．平成13年度と同様の傾向を示し，表中の測定点1から5へと実習室から離れると次第に濃度の低下が観察された．また各フロアのエレベーターホールにおけるホルムアルデヒド濃度も高い時で平均30 ppb台であった．

3. 空気清浄装置の効果評価

解剖実習室には多数の空気清浄装置が設置されているが，その効果についても検討した．その結果，空気清浄装置通過後の排出口の濃度は，吸入口に対する濃度比で平均して高だか6割弱であり，空気清浄装置としてホルムアルデヒドを十分に吸着する機能は保持しえていないことが示された（Table 3）．

4. 自覚症状アンケート調査

自覚症状調査においては，「喉が乾燥する」，「目がチカチカする」，「目がかゆい」，「気分が悪い」，「疲れている」，「集中するのが困難」，「落ち込んでいる」において，普段に比べ解剖学実習室内において有意に高い訴えを認めた（Fig. 3）．

考　　察

本研究の結果は，解剖学実習においては高濃度のホルムアルデヒドに曝露される可能性を改めて示した．現時点ではホルムアルデヒドに関しては作業環境測定の評価のための管理濃度が示されていないので，管理区分を示すことはできない．仮に日本の許容濃度500 ppb（0.5 ppm）[18]を用いて評価すればA測定及びB測定とも第3管理区分となり，作業環境改善などの指導を行う必要があると判断される．

ホルムアルデヒドを取り巻く行政上の対策としては，平成14年春，厚生労働省より「職域における屋内空気中のホルムアルデヒド濃度低減のためのガイドライン」が示され，ホルムアルデヒドなどを製造し，又は取り扱う作業場であって，作業の性質上80 ppb以下とすることが著しく困難な作業場を「特定作業場」とし当面は250 ppbを，それ以外の場所において

は80 ppbを指針値として示している [16]. さらに, 文部科学省の「学校環境衛生の基準」も平成14年4月改訂適用され, 80 ppbの値を基準値としている [19].

また, 実習学生の中には, 目や喉の粘膜刺激症状, 強い臭気, それに伴う倦怠感や気分不良の自覚症状を訴えている者が多くいた. ホルムアルデヒドの急性症状については, 前報にまとめたように上気道および粘膜刺激症状が主となる. 人により感受性は大きく異なるが, のど・鼻および目への刺激閾値はそれぞれ80～2480 ppbおよび480～960 ppb程度と報告されている. また1920 ppbを2連続日40分間曝露された場合, 曝露後24時間までの頭痛なども観察されている [13]. 今回の測定濃度域もこれらに一致し曝露と症状の関連を裏付けるものである.

他大学においても同様の検討がなされ, 大分医科大学においても本学同様に130～1200 ppbの評価が得られている [20]. また福島県立医科大学においても, 実習時の高濃度ホルムアルデヒドとともに眼の刺激症状などの自覚症状を認め, 特にコンタクトレンズ使用者においては高い訴えが認められたという興味ある報告がなされている [21].

欧米においても早くから系統解剖時のホルムアルデヒド曝露に関して, 調査検討が進められ, Skisakは, 個人サンプラーを用いた調査において44%の対象者が1000 ppb以上を示したと報告している [5]. Perkinsらの報告では, やはり個人サンプラーを用いた調査で学生の平均値が1530 ppb, 教員の平均値が1690 ppbであったと報告されている [6]. Akbar-Khanzadehらは解剖学実習において, 今回の調査同様に眼, 喉, 呼吸器の刺激症状とともに努力肺活量, 3秒量の低下などの呼吸器症状を報告し局所排気を含めた換気の改善の必要性を示唆している [9].

本学においても, 平成13年度の測定結果を受けて, 換気状況の調査を実施したところ, 施設の老朽化などに伴い十分な設定換気量が得られていないことが判明した. そこで平成14年度実習期間中に換気装置のフィルター交換などの改修がなされ, 開設当初の時間換気回数10.6回までは届かないものの8.6回まで改善した. しかし, その直後の実習室内のホルムアルデヒド濃度は解剖実習の進展に伴う飛散面積の増加によると思われる理由から前より高値を示した. ピーク時の比較では前年度より3/4程度に低減され換気回数増加の効果は多少認められたが, 学校環境衛生の判定基準を達成するような十分な改善の傾向は認められなかった. 米国においても, 同様に局所排気装置がなく, 時間当り9.8回の全体換気だけの解剖実習室において, 0.635～1.82 mg/m^3(508～1456 ppb相当)とほぼ今回の測定結果と同様の報告がなされている [22]. 解剖実習室は解剖実習台が均等に配置され, 部屋全体に発生源がある状況になっており, 測定値を見ても幾何標準偏差が非常に小さく, 実習室が一様に高濃度を示していることが分かる. こういった環境で, 学校環境衛生の判定基準の80 ppb以下を目指すには今回の実習中の測定値から約10分の1以下に低減しなければならない. しかし, そのためには全体換気だけでは換気回数を現状の10倍以上に高めなければならず現実的に対応するのは困難なことが示唆された.

本学の実習室には, 11台の空気清浄装置が設置されていたが, 残念ながらこれらの清浄機も, これだけ高濃度の発生状況においては十分な除去効果を認められなかった.

近年は, 実習に際しご遺体の固定保存方法も検討が加えられている. 本学では平成13年度の測定以前から, 10%ホルマリン約6 ℓで還流固定した後, 90%アルコールで還流し全身のホルマリンをアルコールに置換した後, 70%アルコールに浸して保存することにより, ホルムアルデヒドの発生の低減に努めている.

しかしこの条件下でも非常な高値を示した.

また,周辺環境への影響に関しては,実習中の学生の出入り時のドアの開閉に伴い解剖実習室のすぐ外の廊下では厚生労働省の示す室内ガイドライン値の80 ppbを超える状況にあるが,廊下では実習室からの距離に応じて低減し,エレベーターホールでは一般環境と相違なかった.また各フロアにおいても上記の80 ppbを超えることはなく良好な環境が認められた.

ホルムアルデヒドの影響評価は,急性の粘膜刺激症状と発がん性が主なターゲットであったが,近年は化学物質過敏症の原因物質としてのホルムアルデヒドの影響が最も懸念されている.解剖実習時のホルムアルデヒド使用に伴う感作に関してはWantkeらが,ホルムアルデヒド特異的IgE抗体の誘導が認められる場合があったが,症状との関連はなかったと報告している [23].化学物質過敏症の本態がいまだ不明な部分が多いため,解剖実習におけるホルムアルデヒド曝露との関連性は評価することが困難なのが現状である.

一方,発がんとの関連に関しては,解剖学実習に従事する教職員・学生や病理学関係者において,鼻腔・頬粘膜上皮あるいは末梢血リンパ球の小核発現や姉妹染色分体交換(SCE)の増加など変異原性試験における陽性結果が多数報告されている [24−27].疫学調査の結果では,まだ確定しないところも多く残されているが,ヒトにおいても鼻咽頭部がん,白血病とくに骨髄性白血病の発生に関連する可能性が最近の報告でもなされている [2−4].

これらのホルムアルデヒドの生体影響を可能な限り予防するためには,原則は発生源対策としてホルムアルデヒドに替わる固定剤があれば理想である.しかし,現実にはホルムアルデヒドが浸透力と固定力に優れ,安価で,最も利用しやすい固定剤である.また,上述のように全体換気での対策では限界がある.Colemanは,各解剖台に局所排気装置を設置し解剖台周辺の空気を吸引し,解剖台に設けた活性炭フィルターにホルムアルデヒドを吸着した後に再び室内に排気するシステムを導入したところ,遺体上20 cmのホルムアルデヒド濃度を30〜90 ppbと大幅に低減することができたと報告している.さらに,解剖台からの排気を室外に行うことで効率よく濃度低減できる可能性を示唆している [28].また,保護具の使用としてマスクとゴーグルを使用することで自覚症状の改善に繋がったという報告もある [29].文部科学省の通知でも,対策の一つとして保護具の有効な利用を提言している [15].しかし,対策としては,まず発生源対策および環境改善に努め,保護具の使用は最終的な対策とすべきである.

結　　論

全国的に同様の測定結果が報告されている中,今後前述のような局所排気装置を導入した解剖台の改修など,抜本的な対策をとらない限り解剖実習におけるホルムアルデヒド濃度の低減は非常に困難であることが示唆された.ただし局所排気装置のついた新規の解剖台の開発・購入は莫大な費用が必要になることが考えられる.本学では,使用者と労働衛生工学の専門家などの共同チームで,既存の解剖実習台に局所排気装置を装着した改善法を開発中でありその成果が期待される.

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Exposure to Formaldehyde during an Anatomy Dissecting Course

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Abstract: Formaldehyde is a flammable, colorless and readily polymerized gas at ambient temperature, and is one of the major pollutants in indoor air. Medical students during their dissection course are exposed to formaldehyde, whose exposure is recently considered to be one of the causes of multiple chemical sensitivity. To understand the system that produces exposures and to plan for implementing control options, this study examined formaldehyde exposures that occurred in the gross anatomy laboratory. Formaldehyde in air was sampled by an active 2,4-dinitrophenylhydrazine(DNPH)-silica gel cartridge, extracted with acetonitrile and analyzed with an high performance liquid chromatograph-ultraviolet(HPLC-UV)detector. The geometric mean formaldehyde concentration was 20～93 ppb in the anatomy laboratory before starting the anatomy dissecting. After beginning the dissecting, however, the highest geometric mean concentrations were 1012～1380 ppb. Significant differences were observed during the exposed period for symptoms of "unusual thirst", "burning eyes", "itchy eyes", "bad feeling", "fatigue", *etc*. in comparison with the non-exposed period. These results show that medical schools should take more concrete measures to reduce exposure to formaldehyde.

Key words: formaldehyde, anatomy dissecting room, symptom, indoor air pollution.

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ELECTROPHYSIOLOGY AND IMMUNOHISTOCHEMISTRY IN THE HIPPOCAMPAL CA1 AND THE DENTATE GYRUS OF RATS CHRONICALLY EXPOSED TO 1-BROMOPROPANE, A SUBSTITUTE FOR SPECIFIC CHLOROFLUOROCARBONS

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Abstract—1-Bromopropane is a newly introduced substitute for specific chlorofluorocarbons whose production was prohibited because of depletion of ozone layers. In this study, we analyzed disinhibitory effects induced by repetitive inhalation of 1-bromopropane for 12 weeks in the hippocampal CA1 and the dentate gyrus. In addition, reversal of the disinhibitory effects was examined 4 weeks after 1-bromopropane inhalation ceased. Exposure rats were placed in a stainless steel inhalation chamber at a concentration of 700 ppm, while the control group was provided only room air in the same type of chamber. Paired-pulse inhibition of population spike was considerably decreased ($P<0.05$) at 5 ms interpulse intervals in the CA1, and at 10 and 20 ms ($P<0.05$) interpulse intervals in the dentate gyrus in slices obtained from exposed rats following 4-, 8- and 12-week inhalation periods. The paired-pulse inhibition was decreased at 5 ms interpulse intervals in the dentate gyrus after 12 weeks of inhalation. These changes were not associated with the paired-pulse ratio of field excitatory postsynaptic potentials, suggesting a reduction of recurrent inhibition. The disinhibition was counteracted with the *N*-methyl-D-aspartate receptor antagonist DL-2-amino-5-phosphonopentameric acid in the dentate gyrus, whereas it was unchanged in the CA1. Tiagabine, a selective inhibitor of GABA transporter GAT1, increased the paired-pulse inhibition in the dentate gyrus, and the increase was less in the exposed rats compared with control rats ($P<0.0003$). The changes in both areas recovered to control levels 4 weeks after cessation of inhalation. Our electrophysiological studies suggest differential and reversible disinhibitory effects in the dentate gyrus and the CA1. 1-Bromopropane-induced disinhibition was further analyzed by immunohistochemical methods. There were no apparent morphological defects in either excitatory or inhibitory neuronal components, supporting the reversibility of physiological changes.

In conclusion, chronic inhalation of 1-bromopropane induces a disinhibition in the CA1 and dentate gyrus that is reversible following cessation of exposure.

Key words: hyperexcitability, intoxication, paired-pulse inhibition, GABAergic system, morphology, inhalation.

The central nervous system (CNS) exhibits vulnerability to artificial chemical compounds (xenobiotic chemicals) through changes in excitability. Recurrent inhibition has been used to test excitability of the hippocampal formation in response to experimental application of xenobiotic chemicals such as alcohol (Durand et al., 1981; Abraham et al., 1981; Steffensen and Henriksen, 1992; Rogers and Hunter, 1992; Criado and Thies, 1994), lindane (Joy and Albertson, 1985; Joy et al., 1995), pyrethroid (Gilbert et al., 1989), triethyltin (Fountain et al., 1988), trimethyltin (Dyer and Boyes, 1984) and dehydroepiandrosterone (Steffensen, 1995).

1-Bromopropane (1-BP; CH_3-CH_2-CH_2Br; CAS No. 160-95-5) and 2-BP (CH_3-$CHBr$-CH_3; CAS No. 75-26-3) were introduced as substitutes for specific chlorofluorocarbons whose production was prohibited because of depletion of ozone layers. 1-BP and 2-BP have been widely used as cleaning agents for metal, precision instruments, electronics, optical instruments, and ceramics. However, because the severe toxicity of 2-BP to humans was confirmed (Kim et al., 1996), the use of 1-BP has been increasing. Recently, effects of 1-BP on the reproductive system and the peripheral nervous system have been reported in animal experiments (Yu et al., 1998; Zao et al., 1999; Ichihara et al., 2000). The reduction of motor nerve conduction velocity and an increase in the latency of tail nerve was reported in rats that inhaled 1-BP of 800 ppm (Ichihara et al., 2000). In human studies, Sclar (1999) reported that a male worker complained of weakness in the lower extremities and right hand, numbness, dysphagia and urinary difficulties following a 2-month exposure to a solvent mainly composed of 1-BP. Ichihara et al. (2002) reported that three female workers who used 1-BP as a solvent with a spray

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Abbreviations: ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; AP5, DL-2-amino-5-phosphonopentameric acid; BP, bromopropane; BSA, bovine serum albumin; CLSM, confocal laser scanning microscope; CNS, central nervous system; CR, calretinin; DG, dentate gyrus; fEPSP, field excitatory postsynaptic potential; FITC, fluorescein isothiocyanate; GABA, gamma-aminobutyric acid; GAD, glutamic acid decarboxylase; MAP2, microtubule-associated protein 2; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; PBS, phosphate-buffered saline; PI, propidium iodide; PS, population spike; PV, parvalbumin; TGB, tiagabine.

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gun in a cushion company, showed abnormal neurological symptoms or signs in the CNS such as headache, dizziness, nausea, insomnia, anxiety, irritation, lightheadedness, confusion, forgetfulness, difficulty in concentrating and listlessness. However, despite increasing use, the effects of 1-BP in the CNS have remained unclear. The authors were the first to demonstrate and report disinhibition in the CA1 and the dentate gyrus (DG) of the hippocampal slice obtained from rats exposed to 1500 ppm of 1-BP vapor (Fueta et al., 2000, 2002c). The granule cell hyperexcitation resulted from an overactivation of *N*-methyl-D-aspartate (NMDA) type glutamate receptor, presumably due to a decrease of GABA-mediated recurrent inhibition. In the present study, we decreased the 1-BP concentration to 700 ppm, close to the levels at which toxicity in the peripheral nervous system was reported. We analyzed disinhibitory effects in the hippocampal CA1 and the DG induced by a repetitive inhalation of 1-BP for 12 weeks, and the reversal of these effects 4 weeks after cessation of inhalation. In addition to the electrophysiological studies, we simultaneously performed morphological analysis to determine whether any structural damage might underlie the 1-BP-induced physiological changes. Because physiological data consistently suggested disinhibitory effects in both CA1 and DG throughout the observation periods, we focused our morphological analysis mainly on the GABAergic neuronal system in these areas. We compared the number of GABAergic interneurons between groups by the disector method that is based on modern stereology. This method enables unbiased, assumption-free counting of cells (Sterio, 1984).

Various aspects of the present study have been reported in abstract or proceedings form (Fueta et al., 2002a,b).

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats (7 weeks of age, 58 rats) were purchased from Kyudo Co., Ltd. (Kumamoto, Japan). For 1 week before inhalation, the rats were kept under the conditions of a 12-h light/dark cycle, controlled temperature (24±1 °C) and humidity (55±5%), and free choice water and food. Rats were divided into control (n=29) and exposure (n=29) groups. Each group was further separated into four groups: 4- (n=6), 8- (n=11) and 12- (n=6) week inhalation, and 4-week clearance after 12-week inhalation (n=6).

Inhalation

The apparatus of the inhalation system was as previously described (Ishidao et al., 2002). 1-BP was obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). The exposure group was placed in a stainless steel inhalation chamber at a 1-BP concentration of 700 ppm, and the control group was provided only room air in the same type of chamber. The temperature of the chamber was kept at 23±1 °C and a light period was from 7 AM to 7 PM. The exposures were performed for 6 h a day, between 9 AM and 3 PM, for 5 days a week from Monday to Friday. Either electrophysiological or immunohistochemical examinations were performed on different rats. However, both types of studies were performed on some of the animals. The experiments were conducted under the control of the Ethics Committee of Animal Care and Experimentation in accordance with The Guiding Principle for Animal Care Experimentation, University of Occupational and Environmental Health, Japan, and the Japanese Law for Animal Welfare and Care (No. 221). All efforts were made to minimize the number of animals used and their suffering.

Hippocampal slice preparation and chemicals

The electrophysiological tests were conducted at the end of the 4th, 8th, and 12th week of exposure, and also at the end of the 4 weeks following cessation of the 12-week inhalation period. There were six rats in each group, with the exception of the 8-week inhalation group, which included seven animals. The rats were deeply anesthetized with diethyl ether after the last exposure of each experimental week. The brain was then gently removed and dipped in an ice-cooled artificial cerebrospinal fluid (ACSF; below 4 °C) saturated with an O_2/CO_2 mixture (95%:5%). The composition of the ACSF in mM was: NaCl, 124; KCl, 2; KH_2PO_4, 1.25; $CaCl_2$, 2; $MgSO_4$, 2; $NaHCO_3$, 26; and glucose, 10. The hippocampi were quickly separated from other brain regions in the cooled stage while being moistened with an ice-cooled ACSF. Then transverse slices of 450-μm thickness were obtained from the middle third region of the bilateral hippocampi with a McIlwain tissue chopper. The slices were transferred to an interface-type recording chamber, which was controlled at 32±0.2 °C, and perfused with ACSF saturated with a mixture of O_2/CO_2 (95%:5%) at a flow rate of 1 ml/min. The slices were perfused with the plasma membrane GABA transporter inhibitor, tiagabine (TGB; 20 μM), and a competitive antagonist of NMDA type glutamate receptors, DL-2-amino-5-phosphonopentameric acid (AP5; 100 μM), prepared from stock solutions.

Stimulation and recordings

Following a 1-hour stabilizing period after slicing, recording glass microelectrodes (1 to approximately 2 MΩ) were placed in the cell layer in the CA1 or DG. Bipolar stimulation electrodes made with stainless wires (50 μm in diameter) were placed on the Schaffer collateral/commissural fibers for CA1 recording or on the perforant path for DG recording. Stimulations consisted of square-wave pulses from a stimulator (SEN7203; Nihon Koden, Tokyo, Japan) via an isolator (Nihon Koden; SS202J). For paired-pulse configuration, the duration of the stimulating current pulse was fixed at 100 μs and the current amplitude was adjusted so as to give a maximum population spike by increasing intensity every 10 μA below 100 μA and about every 100 μA below 1000 μA. Interpulse intervals of the paired-pulse stimulation were 5, 10, 20, 50, 100, 200 and 500 ms for the CA1 and were extended to 1000 ms for the DG. Electrophysiological signals were amplified with a high-impedance amplifier (Axoclamp 2B; Axon Instrument Co., CA, USA) with a bandpass of 10 kHz. The signals were then digitized with an AD converter (Axon Instrument Co.; Digidata 1200) and stored on a computer using P-clamp software (Axon Instrument Co.).

Paired-pulse analysis

The population spike (PS) amplitude and the slope of field excitatory postsynaptic potential (fEPSP) was measured as described in a previous study (Fueta et al., 2002c). For analysis of paired-pulse responses, calculation of the paired-pulse ratio was done as follows:

paired-pulse ratio of PS=2nd PS/1st PS

paired-pulse ratio of fEPSP=2nd fEPSP slope/1st fEPSP slope

Statistical significance was evaluated by the unpaired Student's *t*-test or a repeat measured analysis of variance (ANOVA) for a difference between the 1-BP and control groups. A paired *t*-test was used to examine the difference in the absence and presence of pharmacological manipulation.

Immunohistochemical procedure

Animals for morphological analysis were divided into two groups and exposed to either 1-BP (n=4) or room air (n=4) for 8 weeks under the same conditions as described in the electrophysiological experiments. Four to 6 h after the final exposure to the gases, animals were deeply anesthetized with sodium pentobarbital (10 mg/100 g body weight) and fixed by perfusion through the ascending aorta with a solution containing 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.2 at room temperature. Serial sections transversing the middle third of the hippocampus, 40 μm in thickness, were cut with a vibrating microtome (Leica VT1000; Heidelberg, Germany) and processed for fluorescent-immunohistochemistry as described previously (Fukuda et al., 1998; Fukuda and Kosaka, 2000). Briefly, sections were incubated overnight with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS; pH 7.2), for 5 days with one of following mixtures: (1) rabbit polyclonal antibody (dilution 1:2000) against glutamic acid decarboxylase 67 (GAD67; Chemicon, Temecula, CA, USA; Kaufman et al., 1991) and mouse monoclonal antibody (1:1000) against GAD65 (Chang and Gottlieb, 1988); (2) rabbit antibody (1:5000) against parvalbumin (PV; Kägi et al., 1987) and goat polyclonal antibody S3 (1:2000) which recognizes both GAD isoforms (Oertel et al., 1981); (3) rabbit antibody (1:5000) against calretinin (CR; Swant, Bellinzona, Switzerland), mouse monoclonal antibody (1:10000) against calbindin (Pinol et al., 1990), and goat antibody (1:5000) against neuronal nitric oxide synthase (NOS; Herbison et al., 1996); (4) rabbit antibody (1:50) against glutamate receptor 1 (Chemicon) and mouse monoclonal antibody (1:200) against microtubule-associated protein 2 (MAP2; Leinco Technologies, St. Louis, MO, USA); (5) rabbit antibody (1:100) against synaptophysin (Obata et al., 1987) and mouse monoclonal antibody (1:10,000) against calbindin. Sections were then treated overnight with biotinylated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) against mouse IgG (in cases 1, 3 and 5), goat IgG (in case 2), or rabbit IgG (in case 4). The final step lasting overnight was as follows: (i) in cases 1 and 2 with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG (1:100; Jackson ImmunoResearch) and Rhodamine-X-conjugated streptavidin (1:500; Jackson ImmunoResearch); (ii) in case 3 with FITC-conjugated anti-rabbit IgG, Rhodamine-X-conjugated streptavidin and cy5-conjugated anti-goat IgG (1:500; Jackson ImmunoResearch); (iii) in case 4 with FITC-conjugated streptavidin (1:500; Amersham, Buckinghamshire, UK) and cy5-conjugated anti-rabbit IgG (1:500; Jackson ImmunoResearch); (iv) in case 5 with FITC-conjugated anti-goat IgG (1:100; Jackson ImmunoResearch), Rhodamine-X-conjugated streptavidin and cy5-conjugated anti-rabbit IgG (1:500; Jackson ImmunoResearch). In case 4, propidium iodide (PI; 5 μg/ml) was added to the solution for the last 30 min of the final step. All antibodies were diluted in 1% BSA–PBS containing either 0.3% Triton (cases 2–5) or no Triton (case 1). Sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA) and examined with a confocal laser-scanning microscope (CLSM; MRC 1000; BioRad Hert, UK) equipped with a krypton–argon ion laser and mounted on a light microscope (Optiphoto; Nikon, Tokyo, Japan).

Histological analysis was also performed on brain hemispheres, the contralateral side of which had been used for electrophysiological experiments. These hemispheres were immersed in 4% paraformaldehyde in 0.1 M phosphate buffer immediately after dissection. They were cut with a vibrating microtome and processed for immunocytochemistry in the same manner as described above.

Quantitative analysis of GABA interneurons

The optical disector method using CLSM images (Jinno et al., 1998) was used for the measurement of numerical densities of three subpopulations (NOS, PV, CR) of GABAergic interneurons in the DG and CA1 region. Three rats from both control and 1-BP groups were studied. Serial sections were cut and four of them were selected at random in each rat, processed for immunolabeling as above, and examined in CLSM. Images were taken as a stack at 1 μm step size along z-direction with a ×20 objective (Nikon; N.A.=0.75), zoom factor 1. A rectangle (768×512 pixels) corresponding to the size of 574×383 μm was used for the counting frame, and the stage of the microscope was systematically moved to cover the entire hippocampal formation by a motorized control with step sizes of 594 and 403 μm in the x- and y-directions, respectively. Data were transferred to a personal computer (MacG3) and analyzed by using NIH-Image software (v.1.62). Immunopositive profiles of somata that were contained in the space between the third and twelfth optical slices were counted according to the general counting rule of disector (Sterio, 1984), while the first and second optical slices were set for the guard space and therefore not used for counting. Permeation of antibodies from the section surface was sufficient at least to the 15th optical slices in both control and 1-BP-exposed brains. The numbers of cells located within the DG and CA1 region were summed separately for each region and divided by the area size measured for each region by NIH Image. The anatomical border of each region was easily recognizable by the weak background signals in the immunostainings. Shrinkage factor of 0.80 was used for each direction on a basis of measurements of sizes in some of the sections before and after immunohistochemical preparations. A refraction factor of 1.5 was applied to the size in z-direction. Data were averaged in each animal and then in each group. The calculated numerical densities were statistically compared between groups by Mann-Whitney test.

RESULTS

Paired-pulse analysis

Fig. 1 shows a typical example of paired-pulse responses observed in the CA1 and DG areas of the control and 1-BP-exposed rats after 4 weeks of inhalation. The control rat had a strong paired-pulse inhibition at the 5 ms interpulse interval in the CA1, and at the 10 ms interpulse interval in the DG. On the other hand, almost half of the maximum PS was evoked with the second stimulation in the 1-BP-exposed rat. Paired-pulse profiles of PS in the CA1 and DG of 1-BP-exposed rats were compared with those of control rats (Fig. 2). The increase in paired-pulse ratios of PS at a 5 ms interpulse interval was consistently observed in CA1 through the experimental periods of 4-, 8- and 12-week inhalation (Fig. 2a, b and c). In the DG, the increase in paired-pulse ratios of PS was consistently observed at 10 and 20 ms interpulse intervals for all the experimental periods (Fig. 2e, f and g). In contrast to the PS, there was no difference in paired-pulse ratios of fEPSP between 1-BP-exposed and control rats at the 5 ms interpulse interval in the CA1 and at 5–20 ms interpulse intervals in the DG with the exception of 20 ms interpulse interval at 8-week inhalation (Table 1). Therefore, our results suggest that the disinhibition induced by 1-BP inha-

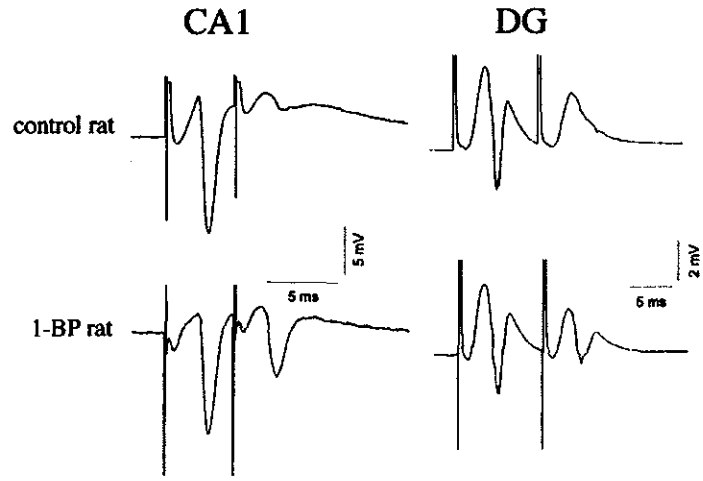

Fig. 1. Examples of paired-pulse PSs recorded from the CA1 and the DG of the control rat and the rat exposed to 1-BP for 4 weeks. Interpulse interval was 5 ms for the CA1 and 10 ms for the DG. Note that the control rat had strong depression of the PS evoked with the second stimulation in both CA1 and DG. On the other hand, the 1-BP-exposed rat had a half maximal PS in response to the second stimulation in both CA1 and DG. The current to induce maximal PS was used for stimulation intensity.

lation is due to a reduction of recurrent inhibition. The decrease in paired-pulse inhibition clearly recovered to control levels both in the CA1 and DG following 4-week suspension of 1-BP inhalation (Fig. 2d and h).

We previously hypothesized that granule cell disinhibition induced by 1-BP vapor is due to an overactivation of NMDA receptors resulting from a reduction of GABAergic recurrent inhibition (Fueta et al., 2002c). A competitive NMDA type glutamate receptor antagonist, AP5, counteracted granule cell disinhibition to the control-like paired-pulse inhibition in the 1-BP-exposed rat(Fig. 3A). In contrast to the DG, neither the paired-pulse profile nor the

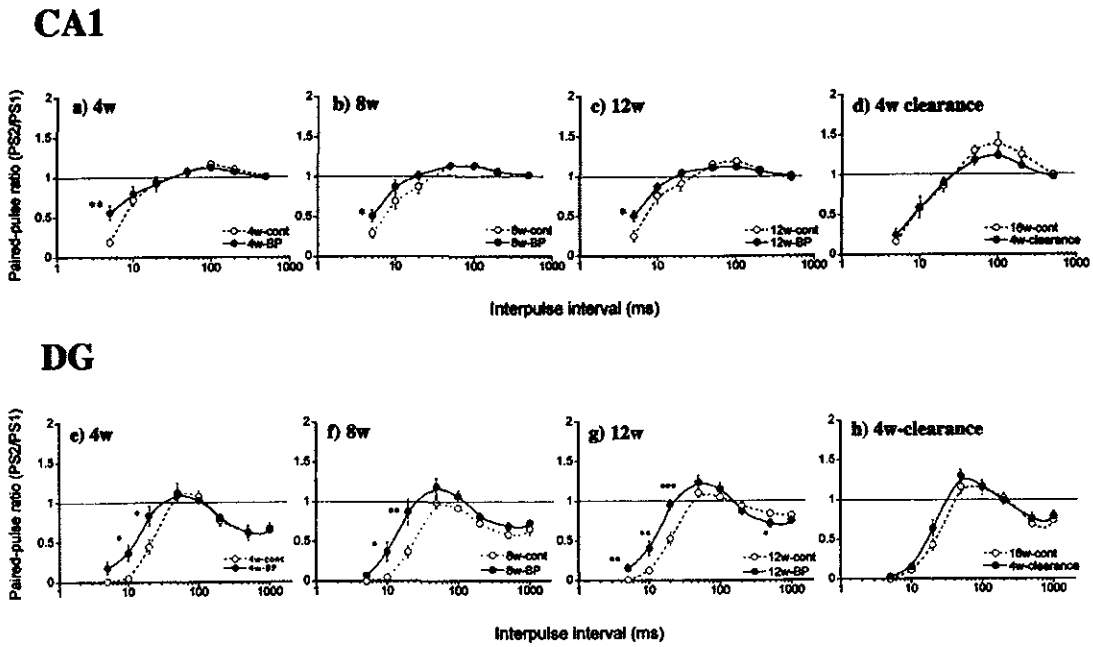

Fig. 2. Paired-pulse profiles of PS in the CA1 and the DG obtained from 1-BP-exposed (●) and control (○) rats at the end of 4-week, 8-week and 12-week inhalation period and at the 4 weeks of suspension following 12-week inhalation. In the CA1, at the short interval of 5 ms, a decrease in paired-pulse inhibition of PS was observed at 4 (a), 8 (b) and 12 weeks (c) of exposure. In the DG, at short intervals (10–20 ms), a decrease in paired-pulse inhibition was observed at 4 (e), 8 (f) and 12 weeks (g) of exposure. The decrease at the shortest interpulse intervals of 5 ms and longer interpulse intervals of 500 ms was evident only for 12 weeks of exposure (g). The change was clearly recovered at 4 weeks of suspension after 12-week inhalation in both the CA1 (*d*) and DG (h). Values are the mean±standard error for seven to 11 slices. (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, by Student's *t*-test.)

Table 1. Paired-pulse ratios of the slope of fEPSP at the short intervals where paired-pulse inhibition of PS was reduced[a]

Subfield	Interpulse interval	Inhalation period					
		4 Weeks		8 Weeks		12 Weeks	
		Control	1-BP exposed	Control	1-BP exposed	Control	1-BP exposed
CA1		n=8	n=10	n=10	n=7	n=12	n=14
	5 ms	0.84±0.25	0.96±0.22	0.88±0.15	0.70±0.16	1.00±0.30	0.92±0.12
DG		n=8	n=6	n=7	n=6	n=12	n=20
	5 ms	0.43±0.05	0.47±0.08	0.50±0.07	0.43±0.08	0.55±0.16	0.50±0.15
	10 ms	0.64±0.07	0.64±0.08	0.65±0.05	0.69±0.08	0.72±0.15	0.68±0.16
	20 ms	0.83±0.05	0.83±0.06	0.80±0.02	0.86±0.05*	0.87±0.14	0.86±0.15

[a] The data are the mean±standard deviation. * P<0.05 compared to control group by Student's t-test.

disinhibition was changed with AP5 application in the CA1 (Fig. 3B).

Activation of synaptic GABA receptors depends on both the concentration and the dwell time of GABA in the synaptic cleft. The GABA transporter plays a crucial role in regulating the dwell time of GABA. Therefore, we applied the GABA transporter inhibitor TGB to increase the dwell time in the synaptic cleft, expecting enhancement of paired-pulse inhibition. As predicted TGB decreased paired-pulse ratios at 10–200 ms interpulse intervals in the 1-BP-exposed and the control rats (Fig. 3C). This indicates that decreased firing of granule cells to the second stimulation is due to an enhancement of inhibitory postsynaptic potentials/currents by TGB. The paired-pulse ratios after TGB application in the control rats were much smaller than those in the 1-BP-exposed rats at 20–200 ms interpulse intervals (repeated measure ANOVA test of TGB data of Fig. 3 between control and 1-BP-exposed rats, P<0.0003). The decreased effects of TGB in the 1-BP-exposed rats suggested that synaptic GABA concentration was reduced in the rats exposed to1-BP vapor.

Morphological analysis

Basic structures of the hippocampus proper and the DG were compared between the control and 1-BP-exposed rats by observing sections prepared for PI staining (Fig. 4A, B). There were no appreciable changes in either the array of principal neurons (pyramidal cells in the hippocampus proper and granule cells in the DG) or distribution of scattered non-principal neurons at each time point of observation.

Detailed morphological features of principal neurons were further investigated in sections immunostained for calbindin and MAP2 (Fig. 4C–E). It was found that the structural integrity of both somata and dendrites of principal neurons in the CA1 region and DG was well preserved after chronic exposure to 1-BP. Profiles showing neuronal cell death such as necrosis, apoptosis, or another very slow type (Fukuda et al., 1999; Wang et al., 1999) were not observed in either group. All these morphological data strongly suggest that chronic inhalation of 1-BP did not lead to apparent loss of principal neurons despite consistent physiological abnormalities.

Synaptophysin-immunoreactive presynaptic terminals and glutamate receptor 1-positive punctate structures were analyzed to detect morphological changes relating to principal neuron synaptic transmission. Comparison of the control and 1-BP-exposed groups again revealed no appreciable differences (data not shown).

Next we compared the distribution patterns of somata and axon terminals of GABAergic interneurons between control and 1-BP-exposed groups (Fig. 5). These structures were immunocytochemically labeled by antibodies against two isoforms of GABA-synthetic enzymes, GAD67 and GAD65. Somata of GABAergic neurons are mainly labeled by anti-GAD67 antibody (Esclapez et al., 1994; Fukuda et al., 1997), whereas their axon terminals are visualized by both anti-GAD67 and anti-GAD65 antibodies (Esclapez et al., 1994) with different laminar profiles (Fukuda et al., 1998). Staining properties of both somata and axon terminals of GABAergic neurons in 1-BP-exposed animals were indistinguishable from those of controls.

Hippocampal GABAergic neurons can be classified into several subpopulations by the presence of different neurochemical markers such as CR, NOS, and PV (Freund and Buzsáki, 1996; Kosaka et al., 1996). Immunocytochemical stainings for these three markers demonstrated that chronic exposure to 1-BP did not change the distribution pattern of GABAergic neuronal subpopulations (Fig. 6). Morphological analysis was also performed on the animals in which paired pulse studies had been conducted, and the same conclusion was obtained as described above for both the excitatory and inhibitory components.

Finally, the numerical density of GABAergic neurons was quantitatively analyzed using the stereological method. As is shown in Table 2, numerical densities of NOS-, CR-, and PV-containing neurons did not decline significantly after 8 weeks' exposure to 1-BP in either CA1 or DG.

DISCUSSION

Disinhibition induced by 1-BP inhalation

In order to study the effects of 1-BP, a substitute for ozone-depleting chlorofluorocarbons, on excitability of a population of neurons, we analyzed paired-pulse ratios of PS and fEPSP evoked in cell layers of the hippocampal CA1 and the DG obtained from 1-BP-exposed rats, and

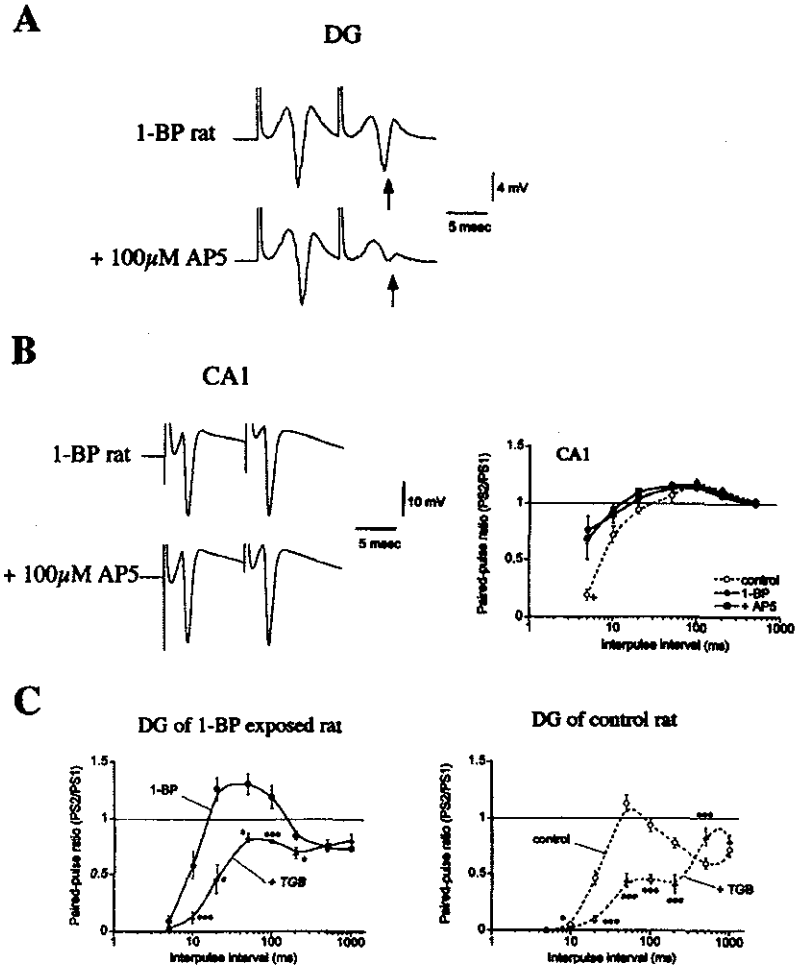

Fig. 3. Effects of NMDA type glutamate receptor antagonist AP5 in the CA1 and the DG and of GABA transporter inhibitor TGB in the DG. (A) AP5 suppressed the PS (arrow) evoked with the second stimulation in the DG obtained from the rat exposed to 1-BP for 12 weeks. (B) In contrast to the DG, AP5 did not change the paired-pulse profile of the CA1. Traces and data of the profile were gathered from 1-BP-exposed rats for 4 weeks. Data of control rats (○) were also from the 4-week group. (C) Effects of TGB on the profiles of the DG in the 1-BP-exposed (●) and control (○) rats. TGB increased the paired-pulse inhibition at 10–200 ms in both exposed and control rats with more intensive suppression in the control. * $P<0.05$, *** $P<0.001$. Significant differences between the presence and the absence of the drug in 1-BP rats were tested by paired *t*-test. The paired-pulse ratios after TGB application in the control rats were much smaller than those in the 1-BP-exposed rats at 20–200 ms interpulse intervals (repeated measure ANOVA, $P<0.0003$).

compared those with control rats. In the present study, repetitive inhalation of 1-BP for 4, 8 and 12 weeks disclosed a decreased paired-pulse inhibition of PS at the 5 ms interpulse interval in the CA1 (Fig. 2A) and at the short interpulse intervals of 5, 10 and 20 ms in the DG (Fig. 3A).These disinhibitory effects are consistent with our previous results obtained in a higher concentration of 1500 ppm, where the disinhibition was more enhanced in both areas (Fueta et al., 2002c). The disinhibition did not seem to be due to a change in feed-forward inhibition of synaptic inputs, since there was no association between paired-pulse ratios of PS and those of fEPSP in our studies at 700 ppm (Table 1) and 1500 ppm (Figs. 5 and 6 of Fueta et al., 2002c). The EPSP/spike curve did not changed even when the paired-pulse ratios of PS increased in both CA1 and DG at 1-week inhalation at the higher concentration of 1500 ppm. It was interpreted from the stimulation/response curves that a subthreshold, a maximal PS and a maximal stimulation were hardly affected by the higher concentration of 1-BP. Therefore, our electrophysiological results suggested that the disinhibition in the CA1 and DG induced by 1-BP inhalation might be due to a reduction of recurrent inhibition.

Differential disinhibition in the DG and CA1

Extracellular paired-pulse configuration has been extensively used to test excitability of the hippocampal formation following xenobiotic exposure (Durand et al., 1981; Abraham et al., 1981; Steffensen and Henriksen, 1992; Rogers and Hunter, 1992; Criado and Thies, 1994; Joy and Albertson, 1985; Joy et al., 1995; Gilbert et al.,

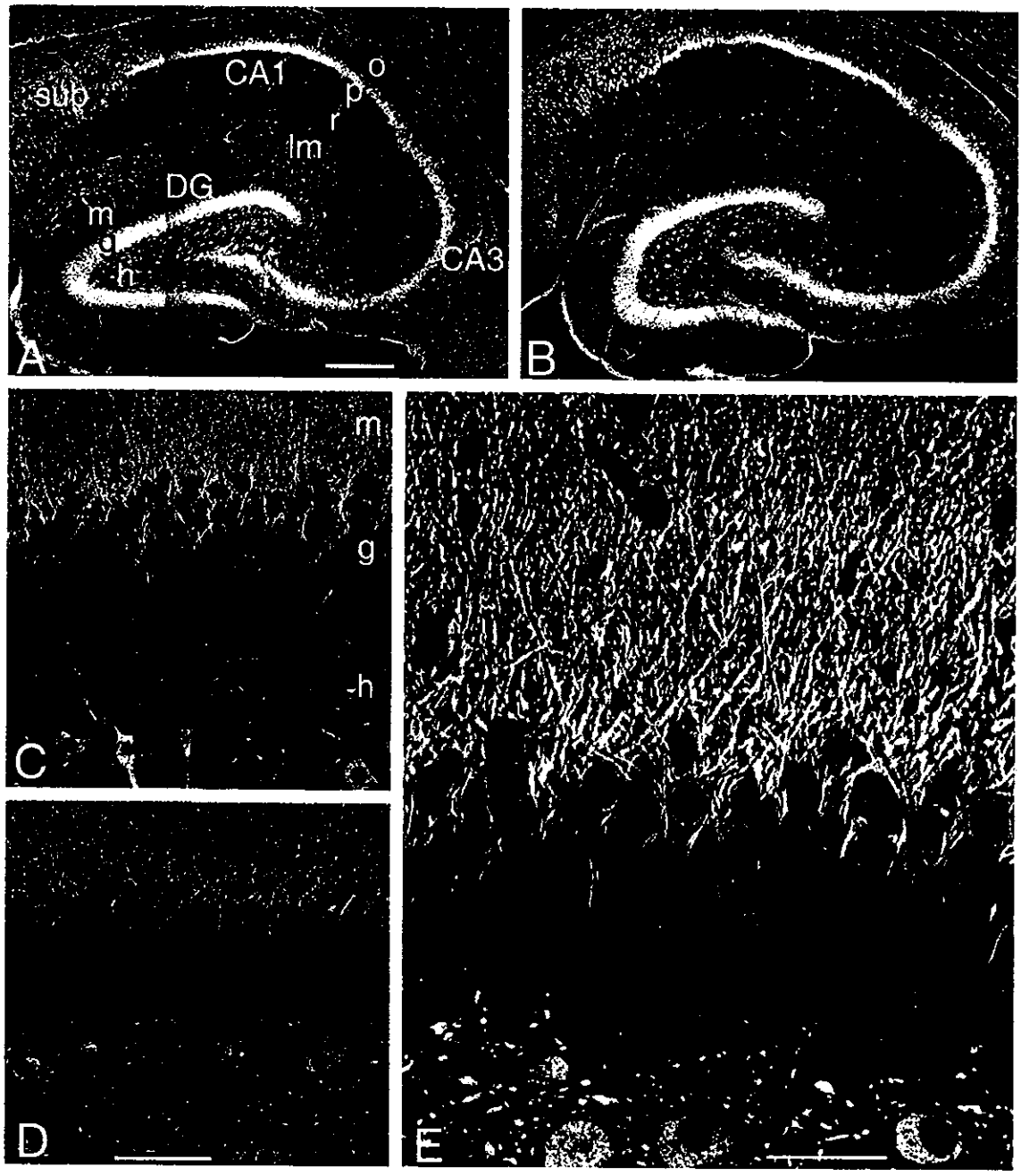

Fig. 4. Cytoarchitecture of the hippocampus proper (CA1 and CA3 regions) and DG of control (A, C) and 1-BP-treated (B, D, E) rats visualized by PI staining (A, B) and MAP2 immunostaining (C–E). (A, B) Montages of CLSM images show that the array of pyramidal cells (p) in the hippocampus proper and that of granule cells (g) in DG was well preserved after 8 weeks' exposure to 700 ppm 1-BP. h, hilus; lm, stratum lacunosum-moleculare; m, molecular layer; o, stratum oriens; r, stratum radiatum. (C–E) MAP2 immunoreactivity is seen in densely packed somata of granule cells and their dendrites ramifying in the molecular layer. Scattered cells in the hilus are also labeled and are assumed to be composed of mossy cells and GABAergic interneurons. Structural features of all these neurons are well preserved after 1-BP exposure, which is further visible in higher magnification (E). Scale bars=500 μm (A, B); 100 μm (C, D); 50 μm (E).

1989; Fountain et al., 1988; Dyer and Boyes, 1984; Steffensen, 1995) as well as seizures (Tuff et al., 1983; Miligram et al., 1991; Psarropoulou et al., 1994; Kapur and Lothman, 1990; Ikeda-Douglas et al., 1998) and ischemia (Chang et al., 1989). Recurrent inhibition was previously discussed in these studies. The duration of recurrent inhibition is likely due to a time course of GABAergic inhibition at the given area, which consists of several sequential steps such as amount released, uptake by transporters at glial cells and presynaptic termi-

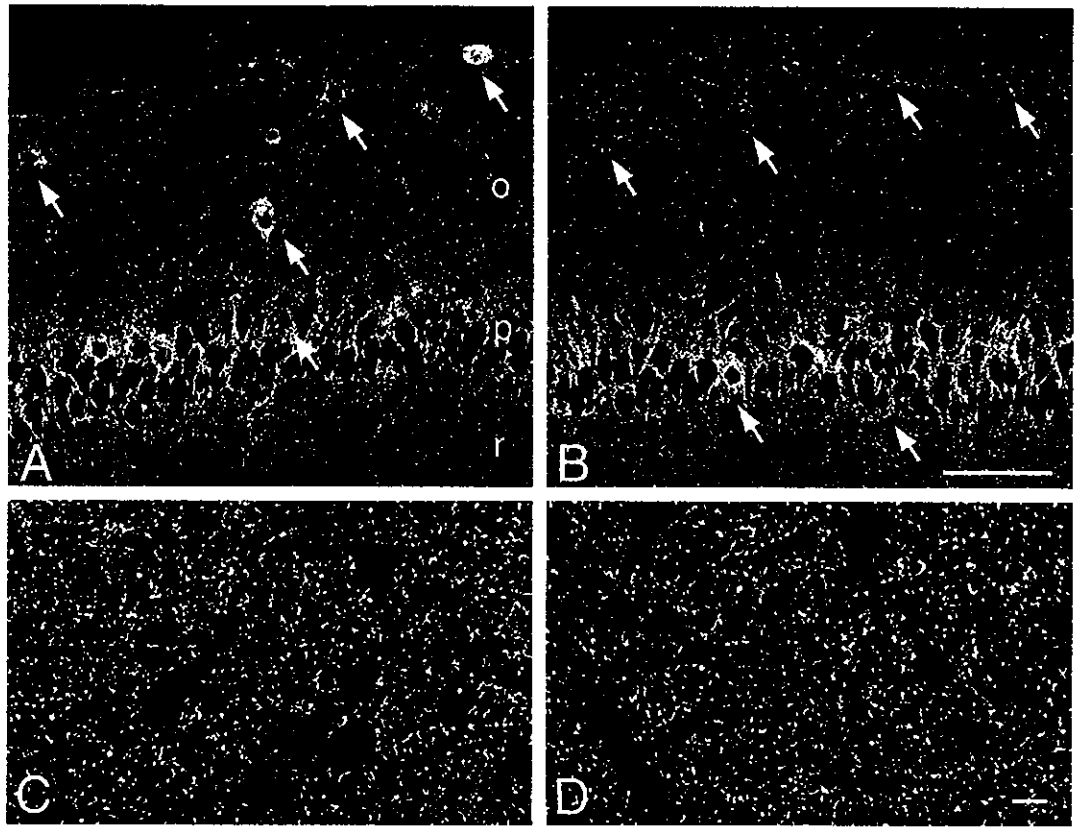

Fig. 5. GAD67 immunoreactivity in the hippocampal CA1 region (A, B) and GAD65 immunoreactivity in the DG (C, D) of control (A, C) and 1-BP-treated (B, D) rats. Arrows indicate somata of GABAergic interneurons labeled by anti-GAD67 antibody. GABAergic axon terminals are labeled as GAD67-immunoreactive numerous punctate structures in the stratum oriens (o), pyramidale (p) and radiatum (r) of CA1 region and as GAD65-immunoreactive puncta in the molecular layer of DG (C, D). There was no apparent change in either GAD67 or GAD65 immunoreactivities after 8 weeks' exposure to 1-BP. Scale bars=100 μm (A, B); 10 μm (C, D).

nals, activation of presynaptic B type autoreceptors, conductance changes mediated with postsynaptic receptors, etc. In order to test if the disinhibition observed in the CA1 and DG of 1-BP-exposed rats is based on a common mechanism(s), we have applied AP5 (50 μM) or the $GABA_A$ receptor enhancer pentobarbital (100 μM) to both areas. In the DG, AP5 suppressed disinhibition (Fig. 3A; also see fig. 7 of Fueta et al., 2002c) and pentobarbital enhanced paired-pulse inhibition in the slices from 1-BP-exposed rats (Fig. 7 of Fueta et al., 2002c). In contrast to the DG, AP5 had no effect on paired-pulse ratios of 10–500 ms interpulse intervals in the CA1 (Fig. 3B), and pentobarbital did not counteract the disinhibition at 5 and 10 ms interpulse intervals (Fig. 1C of Fueta et al., 2002a). The discrepancy of these pharmacological effects in the CA1 and DG suggests that disinhibitory mechanisms induced by 1-BP inhalation may be different in these areas. Our previous results (Tables 1 and 2, and Fig. 2 of Fueta et al., 2002c) showing that the EPSP/PS potentiation, the decrease in the subthreshold of PS, and the stimulation intensity to evoke maximal PSs were observed only in the DG. These findings also suggest differential effects of 1-BP inhalation in the DG and CA1.

Decrease in the GABAergic recurrent inhibition in the DG

The effects of AP5, pentobarbital and TGB on granule cell disinhibition support our hypothesis that a reduction of GABAergic inhibition enhanced activation of NMDA receptors. Granule cell disinhibition could be induced by a reduction of $GABA_A$ receptors (Ono et al., 1997) or activation of presynaptic $GABA_B$ autoreceptors (Misgeld, 1992). TGB application could have increased the dwell time of GABA in the synaptic areas, which would have increased the probability of activating postsynaptic GABA receptors. However, it is not known in our study if it is due to a decrease in GABA release, dysfunction of GABA transporters, overactivation of $GABA_B$ autoreceptors at the terminals, or a difference in the geometric distribution of membrane proteins relating to GABAergic function.

NMDA activation is reported to be involved in paired-pulse facilitation of fEPSP at the slow phase (peaking at 320 ms) in the CA1 (Papatheodoropoulos and Kostopoulos, 1998). However, we did not observe paired-pulse facilitation of fEPSP in either the CA1 or the DG. Overactivated NMDA receptors in the DG observed in our study may have localized mainly in the somatic areas of granule cells. The overactiva-

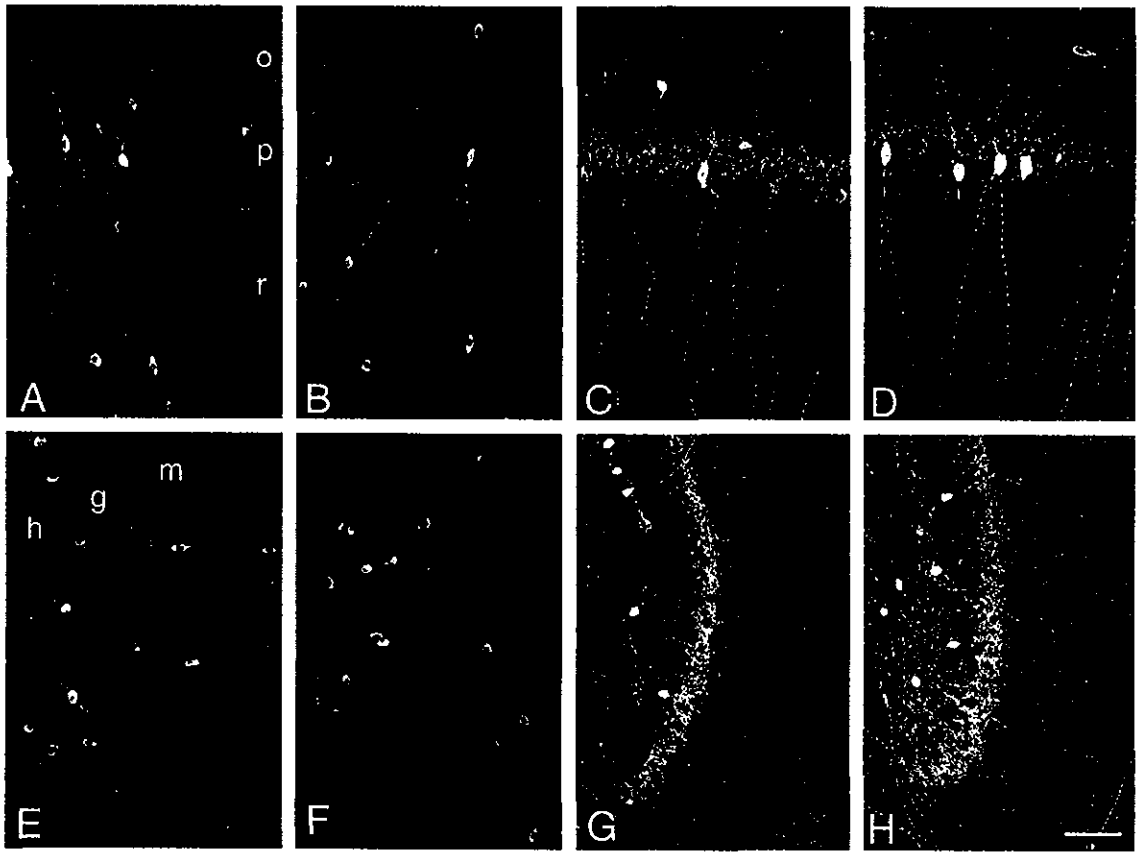

Fig. 6. Distribution of subpopulations of GABAergic neurons in the CA1 region (A–D) and DG (E–H) of control (A, C, E, G) and 1-BP-treated (B, D, F, H) animals, identified by different chemical markers: NOS (A, B, E, F), PV, (C, D), and CR (G, H). Scale bar=100 μm.

tion of NMDA receptors in the DG did not appear to change intracellular signaling such as Ca^{2+}/calmodulin-dependent protein kinase II or mitogen-activated protein kinase in response to 1-BP inhalation (K. Fukunaga and Y. Fueta, unpublished data). Excitation of granule cells is modulated by two populations of GABAergic hilar interneurons. One is recurrent to granule cells, and another is activated by granule cells, but inhibits the recurrent GABA neuron. The hilar mossy cells are in a feedback NMDA receptor-mediated excitatory loop to granule cells. These circuitries could explain why both GABA and NMDA blockers have effects on the disinhibition of paired-pulse responses.

It is not clear how the disinhibition observed in 1-BP-exposed rats could be relevant to neurological disorders observed in workers who were exposed to 1-BP, because they did not exhibit convulsions (Sclar, 1999; Ichihara et al., 2002). However the disinhibition due to a decrease in recurrent inhibition has been found in chronic alcohol treatment (Rogers and Hunter, 1992; Abraham et al., 1981), triethyltin exposure (Fountain et al., 1988), lindane exposure (Joy and Albertson, 1985; Joy et al., 1995) and genetic epilepsy where convulsive episodes had not been experienced (Ono et al., 1997; Fueta et al., 1998), as well as experimental models of epileptic seizures (Tuff et al., 1983; Miligram et al., 1991; Psarropoulou et al., 1994; Kapur and Lothman, 1990; Ikeda-Douglas et al., 1998). From our series of 1-BP inhalation studies and the present results, disinhibition caused by 1-BP inhalation may be described as a predisposing state that results in pathophysiological episodes. However, it is still unclear how1-BP and/or its metabolites directly act on receptors or channels.

Table 2. Cell density of GABAergic interneurons in the CA1 and the DG of control and 1-BP-exposed rats[a]

Cell density (/mm^3) subfield	NOS		CR		PV	
	Control	1-BP exposed	Control	1-BP exposed	Control	1-BP exposed
CA1	864±188	814±126	785±81	746±158	686±145	559±57
DG	1004±82	893±153	524±258	534±148	338±47	283±76

[a] The data are the mean±standard deviation.