Ambulatory activity was decreased by elevation of room temperature ($F(3,56) = 7.0$, $p < 0.01$), but no difference was found between the FMH and control groups ($F(1,56) < 1$, n.s.). Rectal temperature increased significantly in the FMH group at 31 °C on the first day after injection compared to that in the control group ($F(1,56) = 21.9$, $p < 0.01$). FMH had no effect on ingestive behavior or rectal temperature at 21 °C room temperature (not shown in figure).

Discussion

The present study demonstrated an increase in histamine content in the rat hypothalamus at an elevated environmental temperature. FMH is known to be a specific 'suicide' inhibitor of histidine decarboxylase and to decrease neuronal histamine content, specifically and selectively[14,15]. Both previous studies[16,17] and the current study of the saline-treated rats demonstrated that rats can adapt to a change in environmental temperature from 21 °C to 31 °C. Adaptive behavior of rats to 31 °C environmental temperature was attenuated during decrease of endogenous hypothalamic histamine by FMH. These findings imply that increase of hypothalamic histamine content may be important in rat adaptive behavior at high environmental temperature.

Our previous studies demonstrated that neuronal histamine acted through H1-receptors in the hypothalamus to suppress food intake, and changes in hypothalamic histamine content might modulate daily fluctuation of food intake[18–21]. Together with these findings, the present results verify one of the physiological functions modulated by brain histamine.

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1 Hamilton, C. L., in: Handbook of Physiology: Section 6: Alimentary Canal, vol. 1, p. 303. Ed. C. F. Code. American Physiological Society, Washington DC 1967.
2 Cormarèche-Leydier, M., Pflügers Arch. *389* (1981) 171.
3 Hori, T., and Tsuzuki, S., Pflügers Arch. *390* (1981) 219.
4 Leibowitz, S. F., Brain Res. *63* (1973) 440.
5 Kraly, F. S., Nature *302* (1983) 65.
6 Clineschmidt, B. V., and Lotti, V. J., Archs int. Pharmacodyn. *206* (1973) 288.
7 Brezenoff, H. E., and Lomax, P., Experientia *26* (1970) 51.
8 Cox, B., Green, M. D., and Lomax, P., Experientia *32* (1976) 498.
9 Glowinski, J., and Iversen, L. L., J. Neurochem. *13* (1966) 655.
10 Yamatodani, A., Fukuda, H., Wada, H., Iwaeda, T., and Watanabe, T., J. Chromat. *344* (1985) 115.
11 Nohta, H., Mitui, A., and Ohkura, Y., Analyt. chim. Acta *165* (1984) 171.
12 Sakata, T., Fukushima, M., Tsutsui, K., Arase, K., and Fujimoto, K., Physiol. Behav. *28* (1982) 641.
13 Fujimoto, K., Sakata, T., Shiraishi, T., Kurata, K., Terada, K., and Etou, H., Am. J. Physiol. *251* (1986) R481.
14 Kollonitsch, J., Patchett, A. A., Marburg, S., Maycock, A. L., Perkins, L. M., Doldouras, G. A., Duggan, D. E., and Aster, S. D., Nature *274* (1978) 906.
15 Maeyama, K., Watanabe, T., Taguchi, Y., Yamatodani, A., and Wada, H., Biochem. Pharmac. *31* (1982) 2367.
16 Sakata, T., Fukagawa, K., Fujimoto, K., Yoshimatsu, H., Shiraishi, T., and Wada, H., Experientia *44* (1988) 216.
17 Hamilton, C. L., and Brobeck, J. R., Am. J. Physiol. *207* (1964) 291.
18 Fujimoto, K., Sakata, T., Ishimaru, T., Etou, H., Ookuma, K., Kurokawa, M., and Machidori, H., Psychopharmacology *99* (1989) 257.
19 Sakata, T., Ookuma, K., Fukagawa, K., Fujimoto, K., Yoshimatsu, H., Shiraishi, T., and Wada, H., Brain Res. *441* (1988) 403.
20 Fukagawa, K., Sakata, T., Shiraishi, T., Yoshimatsu, H., Fujimoto, K., Ookuma, K., and Wada, H., Am. J. Physiol. *256* (1989) R605.
21 Ookuma, K., Yoshimatsu, H., Sakata, T., Fujimoto, K., and Fukagawa, K., Brain Res. *490* (1989) 268.

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The influence of cold or isolation stress on resistance of mice to West Nile virus encephalitis

D. Ben-Nathan[a] and G. Feuerstein[b]

[a] *Department of Virology, Israel Institute for Biological Research, P. O. Box 19, 70450 Ness-Ziona (Israel), and* [b] *Department of Pharmacology, Smithkline Beecham, P. O. Box 7929, L-524, Philadelphia (Pennsylvania 19101, USA)*
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Summary. The effect of cold or isolation stress on mortality rate and brain virus level were investigated in mice infected with West Nile virus (WNV). Exposure of mice for 5 min/day to cold water (1 ± 0.5 °C) for 8–10 days resulted in 92% mortality as compared to 47% in control mice ($p < 0.001$). Mice housed in individual cages (isolation stress) were also more susceptible to WN viral infection, as shown by increased mortality rate reaching 85% as compared to 50% in mice housed 6 per cage ($p < 0.01$). Cold or isolation stress increased blood brain and spleen virus levels as early as 2 days after inoculation. After 8 days of isolation or cold stress, mice inoculated with WNV had 8.9 and 9.0 $\log_{10}$ plaque forming units in the brain, respectively, as compared to 6.9 in the control ($p < 0.01$–0.001). Furthermore, lymphoid organs such as spleen and thymus showed severe mass loss. These data suggest that physical or non-physical stress situations enhance WNV encephalitis by accelerating virus proliferation and increase mortality in mice.

Key words. West Nile virus; stress; isolation; cold stress; encephalitis.

West Nile virus (WNV) is a member of the flavivirus genus within the flaviviridae family[1] which is widely distributed throughout Asia, Africa and north of Europe[2]. WNV is a neurotropic arbovirus[3] which is capable of endemic spread[4,5]. Wild birds are the primary host but high antibody rates in a variety of animals including man indicate a broad infectious spectrum. In man, the morbidity rate is low and severe cases of encephalitis occur only occasionally, but subclinical infections are common[6]. In parts of Africa, up to 70% of the human population may possess antibodies[6].

Since convalescence from WNV encephalitis is dependent on the humoral immune response[7], antibody formation by an intact immune system is of primary importance. Therefore, factors which interfere with the integrity of the immune system might affect the ability to respond adequately to viral infections, including WNV encephalitis.

Environmental or physical stress conditions are known to affect the immune system. For example, starvation[8] can cause involution of the thymus, spleen and lymph nodes through activation of the pituitary adreno-cortical axis[9-11]. Further, interferon production is suppressed in stress situations[12], and reduced numbers of T-helper lymphocytes were found under various types of stress[13,14]. In fact, stress paradigms like conditioned avoidance behavior have already been shown to reduce resistance to several infectious agents, including herpes simplex[15], Coxsackie$_{B1}$[16], and vesicular stomatitis[17] virus. The following studies explore the interaction of physical cold and social isolation variables on the course of WNV encephalitis, including viremia, brain virus levels and mortality. The WNV has been chosen as a model for stress interaction with viral diseases since it provides a most reproducible and predictable model in mice.

Materials and methods

Virus. The original strain of virus was isolated from a human case of WNV infection[5]. The virus stock was prepared and assayed in Vero cells in our laboratory. The virus stock used for the experiments contained 3×10^8 plaque forming units (PFU/ml). The intracerebral titer (LD_{50}) was 1.3×10^7/ml and 6.9×10^6 mouse i.p. LD_{50}/ml.

Mice. Female Charles River mice CrL: CDR-1 (ICR) BR were obtained at the age of 21 days (10–12 g b. wt) and kept in our vivarium until the age 27–42 days. In all the studies, mice of the same age and batch were used in each separate experiment.

Virus inoculation. Each mouse was inoculated with 0.1 ml (i.p.) of WNV containing 20–200 PFU. The virus dilutions were performed using inactivated rabbit serum (10%) in 0.9% saline containing penicillin (1000 µg/ml). Groups of 6–18 mice were used and the results were calculated according to the method of Reed and Muench[18].

Stress. Cold stress: Mice were placed for 5 min/day in cold water (1 ± 0.5 °C). The mice could stand in the water which was 3 cm deep. WNV was inoculated and immediately thereafter the mice were exposed to the cold stress. Stress continued every day until 8–10 days post inoculation. For mortality rate, mice were observed until the end of the experiment (21 days) and for brain virus level, mice were sacrificed at 4, 5, 6, 7, and 8 days after inoculation.

Isolation. Mice were housed in individual cages soon after inoculation until the end of the experiment or sacrificed for brain virus level. Crowded mice were housed in groups of 12 or 18 per cage soon after inoculation until the end of the experiment. Control mice were housed 6 per cage. All mice received the same dose of WNV.

Tissue cultures. Vero cells: The Vero cell line was derived from kidneys of a normal African green monkey. The cells are grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum (FCS).

BHK cells: A baby hamster kidney (BHK) cell line is grown in Eagle's F-12 medium supplemented with 10% tryptose broth and 10% calf serum.

Isolation of WNV from the spleen and brian of infected mice. Each brain or spleen was rinsed in cold PBS and sonicated and the virus suspension was centrifuged at 3000 rpm for 10 min. The supernatant was aliquoted into plastic tubes and stored at −70 °C until further processed. In experiment using pools of brains, six brains from each group were pooled for each assay to determine brain virus level. The virus level in the blood, brain or spleen was determined by titration of virus in Vero cells or in BHK cell line.

Organ weight. Mice were individually weighed at 7 days after inoculation. The mice were sacrificed and the thymus and spleen immediately removed and weighed aseptically. The spleens and brains of each group were individually rinsed in cold PBS (1 ml per spleen and 2 ml per each brain) containing 2% FCS and penicillin (1000 µg/ml).

Titration of virus in tissue cultures. For demonstration of WNV plaques in Vero cells, the original plaque technique of Dulbecco and Vogt[19] was used. A dilution of virus is added to Vero cells monolayers in petri dishes and incubated at 37 °C for 1 h to permit viral absorption. The monolayer is overlaid with MEM × 2 and tragacanth (Gum tragacanth Grade III G-1128, Sigma) containing 2% FCS and 2.4% $NaHCO_3$. The cultures were incubated (37 °C, 5% CO_2) for 72 h. Plaques are counted after staining the monolayer with neutral red (0.05). The same procedure was followed for BHK cells. All plaques were counted by an investigator unfamiliar with the coded samples.

Data analysis. All data in text and figures are mean values ± SEM for the indicated number of mice. Data were analyzed by ANOVA and the Student-Newman-Keul test for a-posteriori multiple comparisons or the Kruskal-Wallis followed by Mann Whitney U-test were appropri-

ate. The Fisher exact probability test was used for survival analysis. Data were considered significant for $p < 0.05$.

Results

Dose response for WNV-induced mortality in mice. In initial experiments, the dose of WNV that caused mortality of about 50% was determined in mice 3–4 weeks old. For determination of LD_{50}, mice were inoculated with 0.1 ml per mouse with the following PFU: 5, 50, 500, 5000; 6 mice were used for each virus dilution. The mortality rate for the respective PFU's were 16, 50, 67, and 100%, respectively.

Effect of cold stress on mortality of mice inoculated with WNV. The cold stress paradigm used in this study was chosen from preliminary experiments where mice were placed for 5 and 10 min in cold (1 ± 0.5 °C) water (see Methods). The 5-min time period was selected since no reduction in body weight was seen in mice exposed to this cold stress. The effect of cold stress on the mortality rate of mice inoculated with 50 PFU of WNV is shown in table 1. The results show that exposure of WNV-inoculated mice to cold stress markedly increased the mortality as compared to control mice. In mice that were exposed to cold stress for 5 days before virus inoculation, the mortality rate was 92% while mice infected with WNV and exposed to cold stress on the same day showed a mortality rate of 88%; both groups had a mortality rate much higher than the control group 47% ($p < 0.001$). Signs of illness in stressed mice were first observed 6–7 days after infection, as evidenced by the ruffled fur and depressed activity. In controls, these signs appeared first at 8–9 days.

Table 2 shows the overall mortality rate of mice inoculated with 20 PFU or 200 PFU per mouse. Again, exposure of mice to cold immediately after virus inoculation increased the mortality to 75% and 100% as compared to control mice which had 25% and 58% mortality after 20 and 200 PFU, respectively. Inoculation of the virus 5 days after the onset of cold exposure also increased the mortality to 92% at 20 PFU and 100% at 200 PFU. Furthermore, mice exposed to cold stress for 5 days and inoculated with 20 PFU on the 5th day showed 66% mortality at day 9 as compared to 0% in the non-stressed mice at the same day ($p < 0.01$).

The effect of isolation or crowding stress on mortality of mice infected with WNV. These experiments were done in mice inoculated with 50 PFU. Table 3 shows that mice housed in single cages had 85% mortality rate as compared to 52% in mice housed 6/cage. Mice housed 12 or 18 per cage also showed an increased overall mortality rate of 58% and 65%, respectively.

In the group experiments (average of 6, 4 and 5 experiments, respectively), mice were inoculated with 20 PFU per mouse (fig. 1). In the individual experiments ($n = 6$), mice were inoculated with 150 PFU per mouse. In the group experiments, control mice showed virus titer of 6.9 ($\log_{10}$ PFU), whereas the virus titers in isolated or cold-stressed mice were 8.7 and 8.6 ($\log_{10}$ PFU), respectively ($p < 0.01$) (fig. 1). In the individual experiments, virus titer of mice in the control group was 5.7 ($\log_{10}$ PFU), in isolated mice 8.5 and in cold stress 9.2 ($\log_{10}$ PFU)

Table 1. The effect of cold stress on the mortality rate of mice inoculated with 50 PFU of WNV

Treatment Group	Experiment 1		Experiment 2		Experiment 3		Total	
	D/T	%	D/T	%	D/T	%	D/T	%
Cold only *	0/6	0	0/6	0	0/6	0	0/6	0
Control	7/12	58	6/12	50	4/12	33	17/36	47
Cold $D_0 + Vd_0$	12/12	100	9/12	75	ND		21/24	88*
Cold $D_0 + Vd_5$	ND		12/12	100	12/14	86	24/26	92**

* 5 min a day at 1 ± 0.5 °C; D/T = dead/total; ND = not determined; * $p < 0.05$; ** $p < 0.01$; D_0 = start of cold stress; Vd_0 = inoculation on D_0; Vd_5 = inoculation of virus at D_5 of stress.

Table 2. The effect of cold stress on mortality of mice inoculated with 20 or 200 PFU of WNV

Treatment group	D/%D	Overall mortality rate by day					
		7	8	9	10	11	12
Control	Dead			0	1	1	1
(20 PFU/mouse)	% mortality			0	8	17	25
Control	Dead			2	1	4	0
(200 PFU/mouse)	% mortality			17	25	58	58
Cold $d_0 + Vd_0$	Dead		1	2	3	2	1
(20 PFU/mouse)	% mortality		8	25	50*	67+	75*
Cold $d_0 + Vd_0$	Dead		2	4	2	4	0
(200 PFU/mouse)	% mortality		17	50	67	100+	100+
Cold $d_0 + Vd_5$	Dead	1	3	4	0	3	0
(20 PFU/mouse)	% mortality	8	33	66*	66*	92*	92*
Cold $d_0 + Vd_5$	Dead	3	1	2	2	3	1
(200 PFU/mouse)	% mortality	25	33	50	67	92+	100+

* $p < 0.01$ from the control group. + indicates statistical significance vs 20 PFU group at $p < 0.05$. Number rounded to nearest digit. N = 12 in each group. % mortality is cumulative. D/D% = number of dead mice/percent of dead mice.

Table 3. The effect of isolation or crowding stress on the mortality of mice inoculated with West Nile virus

Mortality rate (% of dead mice) Mice/Cage	Experiment 1 D/T	%	Experiment 2 D/T	%	Experiment 3 D/T	%	Experiment 4 D/T	%	Experiment 5 D/T	%	Total D/T	%
1	10/12	83	9/12	75	10/12	83	11/12	92	6/6	100	46/54	85*
6	5/12	42	5/12	42	6/12	50	12/18	67	3/6	50	31/60	52
12	12/24	50	4/12	33	18/24	75	8/12	67	ND		42/72	58
18	ND		6/18	33	14/18	78	14/18	78	ND		34/54	63

* $p < 0.01$; compared to normal housing (6/cage). ND = not determined. D/T = dead/total mice. % = percent from total.

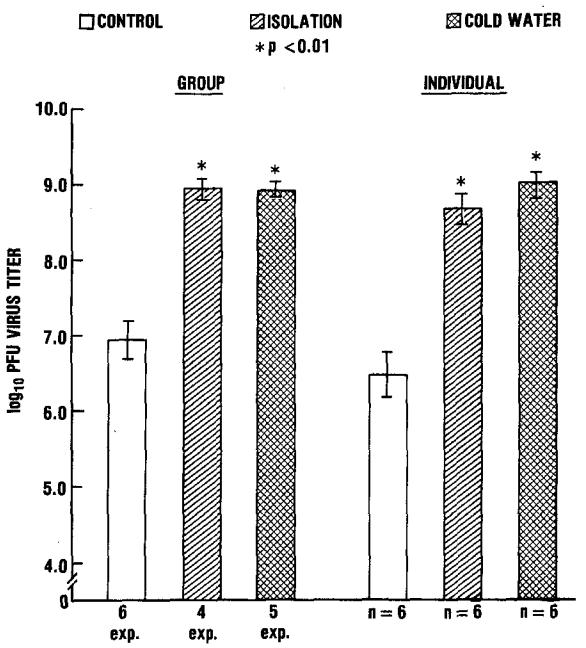

Figure 1. The effect of isolation or cold stress on brain WNV levels 8 days after inoculation. Blank columns represent virus levels in WNV inoculated mice which were not subjected to stress. Matched bars represent mice exposed to isolation stress while double hatched bars represent mice exposed to cold water stress. Asterisk denotes statistical significance from non-stressed mice. Group study represents mice brains pooled for the virus assay while the individual study represents assay performed on each individual mice brain.

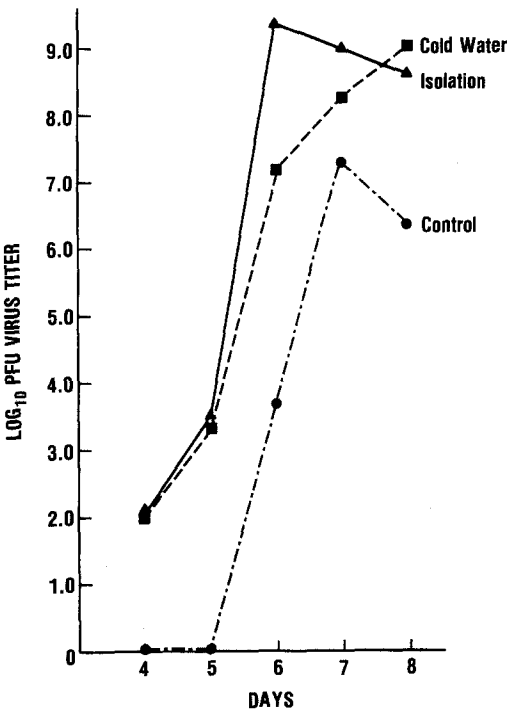

Figure 2. Ontogenesis of brain WNV of mice inoculated with 10 PFU. ●—·—·—●: control mice (non-stressed); ▲——▲: mice exposed to isolation; ■———■: mice exposed to cold stress. Ordinate denotes virus level in the brain at different days after inoculation. All virus levels in the stressed mice brain are significantly higher ($p < 0.01$) than those of the non-stressed group.

($p < 0.01$). The time course of virus detection in the brain for control and stressed mice is given in figure 2. The data clearly show that both stress paradigms resulted in high titers of virus in the brain at a time when no virus could be detected in the non-stressed animals.

Table 4 demonstrates spleen and brain WNV levels in control, isolated and cold-stressed mice 7 days after WNV inoculation (20 PFU/mouse). The data clearly show significantly higher virus levels in both organs.

Table 5 shows the level of WNV in the blood of control (non-stressed), cold-stressed or isolated mice infected with WNV, 20 PFU/mouse. The data show viremia on day 2, 3 and 4 after inoculation (6 mice from each group at each day). Both isolated and cold-stressed mice had higher blood virus levels; moreover, the proportion of mice found positive for viremia (over 20 PFU/ml) was also significantly higher in the stressed animals as compared to the controls.

Effect of isolation or cold stress on spleen, thymus and body weight. Table 6 provides the weight of the thymus, spleen and body of mice inoculated with WNV 20 PFU/mouse, and exposed to isolation or cold stress. WNV inoculation of normal mice had no significant effect on spleen or body weight but significantly reduced the thymus weight. Cold or isolation stress alone significantly reduced spleen weight by 43% and 40%, respectively and thymus weight by 55% and 53%, respectively. However, cold stress did not significantly change body weight while isolation retarded the growth by 20%. The combined effect of isolation or cold stress with the WNV tended to further reduce the lymphoid organ weight without further reduction of body weight.

Table 4. The effect of cold isolation stress on spleen and brain WNV level

Treatment group	$\log_{10}$ Spleen	PFU/organ Brain
Control	2.2 ± 0.5	5.8 ± 0.5
Cold	3.8 ± 0.2*	8.8 ± 0.7*
Isolation	3.8 ± 0.1*	8.6 ± 0.6*

* $p < 0.01$ compared to control. 6 mice were used in each group. Cold stress was introduced on the day virus inoculation.

Table 5. The effect of cold or isolation stress on blood virus levels

Treatment group	PFU/ml	P/T
Control	51 ± 22	6/18
Cold	419 ± 173**	16/18*
Isolation	670 ± 394*	16/18*

* < 0.005; ** $p < = 0.001$; P/T = positive (> 20 PFU/ml)/total. Statistical analysis was done by the Kruskal-Wallis and Mann-Whitney U-test. Cold stress was introduced on the day of virus inoculation.

Table 6. The effect of cold or isolation stress on spleen, thymus and body weight of mice 7 days after WNV inoculation

Treatment group	Spleen (mg)	Thymus (mg)	Body weight (g)
Control	130.2 ± 6.5	85.6 ± 3.5	21.0 ± 0.4
WNV	114.9 ± 12.6	56.2 ± 6.1**	21.3 ± 1.1
Cold	74.8 ± 4.1**	38.1 ± 3.4***	19.0 ± 0.4
Cold + V	55.7 ± 5.2**	30.0 ± 2.0***	17.5 ± 0.6*
Isolation	78.6 ± 18.8**	40.3 ± 9.7***	16.9 ± 2.1*
Isolation + V	39.8 ± 4.0***	16.2 ± 1.5***	15.6 ± 0.6*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared to control.
V = Virus. Six mice were used in each experimental group. Cold stress was introduced on the day of virus inoculation.

Discussion

Our results show significant differences in mortality rates from WNV infection between stressed and non-stressed mice. Exposure of mice to cold or isolation stress increased the mortality from 47% in control to 85–100% in the various stress paradigms. Furthermore, cold stress accelerated the appearance of symptoms and death.

The increased mortality in stressed mice was associated with higher levels of WNV in the brain which exceeded 100-fold of the levels found in non-stressed mice. Furthermore, stressed mice showed increased viremia along with higher levels of WNV in the spleen.

While the mechanisms involved in the higher titer of viruses, organ virus levels and mortality of stressed mice are still obscure, it is suggested that these stressors interfere with immune responses pertinent to the viral infection. Both isolation and cold-stress resulted in substantial involution of lymphoid organs which are primarily responsible for B cell (spleen) and T cell (thymus) mediated responses. Thus, each of the stress paradigms caused significant reduction in the spleen and thymus mass; the involution of these organs was not augmented by the viral infection although some tendency to further reduction of spleen and thymus weight were noticed in isolated mice infected with WNV. These data indicate that a typical stress response has been established during isolation or cold exposure as previously shown in mice exposed to avoidance learning stress paradigms.

Preliminary data from our laboratory indicate that plasma levels of corticosterone in WNV-inoculated mice which were exposed to cold stress are higher than the levels of normal of WNV-infected mice (unpublished observations). Furthermore, antibodies against WNV are not detected throughout the first 2 weeks following WNV inoculation. Therefore, it is conceivable that the stress situations interfere with other cellular or humoral immune responses.

Similar findings were reported in mice housed alone and infected with *M. tuberculosis*[20] or encephalomyocarditis[21]; corticosteroid levels were higher in mice housed individually than in those housed in groups. To our knowledge, there are no other studies that have considered the effects of individually housing or cold stress on mortality of mice infected with WNV.

Similar susceptibilities to viral infections have been reported in mice exposed to a variety of stress situations. Escalation of the viral infection was shown in mice exposed to high intensity sound after inoculation with vesicular stomatitis virus[17] and in mice subjected to avoidance learning stress after exposure to herpes simplex[15], $Coxsackie_{B1}$[16] or vesicular stomatitis virus[22]. These stress paradigms were shown to inhibit cellular immunity[23,24] and hyper-secretion of adreno-corticosteroids[10] as shown in rats subjected to cold stress, immobilization[25] or isolation[21]. Corticosteroids can affect the immune system[26] by impairing interferon production[27], reduction in T-helper cell level[28], increased T-suppressors[29], inhibition of natural killer cell production[30], and fibroblast growth[31].

Since T cells are important in recovery from acute viral infections[32–35] and are the main source of interferon production, the stress situations used in this study might have diminished the T-cell-mediated immune response. This possibility is supported by studies indicating that the inflammatory response is immunologically specific and dependent on T cells[7,36–38].

Arboviral infections are characterized by early viremia which is followed by early multiplication of the virus in extraneural tissues with subsequent spread to the brain[39]; variation in the extraneural phase of infection is an important determinant in the occurrence of CNS invasion[40]. Therefore, our results indicate that extraneural virus replication may be more widespread in stressed mice. This mechanism of invasion might be facilitated following trauma or in dual infection[40,41].

In summary, our data suggest that cold or isolation stress may influence cellular and humoral immunity. This leads to increased susceptibility to viral infection and enhanced viral invasion of the CNS. Furthermore, investigation of the effects of cold or isolation stress on the immune system is warranted.

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1 Forterfield, J. S., Casals, J., Chuma Kov, M. P., Gaidamovich, S. Y., Hannoun, C., Holmes, T. H., Horzinek, M. C., Mussgay, M., Okerbloom, N., Russel, P. K., and Trent, D. W., Intervirology *9* (1978) 129.
2 Work, T. H., Am. J. trop. Med. Hyg. *20* (1971) 169.
3 Weiner, L. P., Cole, G. A., and Nathanson, N., J. Hyg. (*conb*) *68* (1970) 435.
4 Hayes, C. G., Bagar, S., Ahmend, T., Chowdhry, M. A., and Reisen, W. K., Trans. Roy. Soc. trop. Med. Hyg. *76* (1982) 431.
5 Goldblum, N., Sterk, V. V., and Paderski, B., Am. J. Hyg. *59* (1954) 89.
6 Chamberlain, R. W., in: The Togaviruses Biology, Structure Replication, p. 175. Ed. R. W. Schlesinger. Academic Press Inc., New York 1980.
7 Johnson, R. T., J. infect. Dis. *155* (1987) 359.
8 Ben-Nathan, D., Heller, E. D., and Perek, M., Poult. Sci. *56* (1977) 1468.
9 Parillo, J. E., and Fauci, A. S., Am. Rev. Pharmac. Toxic. *19* (1979) 279.
10 Selye, H., The Physiology and Pathology of Exposure to Stress. Montreal 1950.
11 Friedman, S. B., and Glasgow, L. A., Pediat. Clin. N. Am. *13* (1966) 315.
12 Jensen, M. M., Proc. Soc. exp. Biol. Med. *142* (1973) 820.
13 Kiecolt-Glaser, J. K., Speicher, C., Holliday, J. E., and Glaser, R. J., J. Behav. Med. *7* (1984) 1.
14 Sapse, A. T., Med. Hypotheses *13* (1984) 31.
15 Rasmussen, A. F., March, J. T., and Brill, N. O., Proc. Soc. exp. Biol. Med. *96* (1957) 183.
16 Johnson, T., Lavender, J. F., Multin, E., and Rasmussen, A. F., J. Immun. *91* (1963) 569.
17 Jensen, M. M., and Rasmussen, A. F., J. Immun. *90* (1963) 21.
18 Reed, L. G., and Muench, M., Am. J. Hyg. *27* (1938) 493.
19 Dulbecco, R., and Vogt, M., J. exp. Med. *99* (1956) 167.
20 Tobach, E., and Bloch, M., Am. J. Physiol. *187* (1956) 399.
21 Friedman, S. B., Glasgow, L. A., and Adir, R., Psychosom. Med. *32* (1970) 285.
22 Yamada, A., Jensen, M. M., and Rasmussen, A. F., Proc. Soc. exp. Biol. Med. *116* (1964) 677.
23 Wistor, R., and Hildeman, W. H., Science *131* (1960) 159.
24 Chang, S. S., and Rasmussen, A. F., Nature *205* (1965) 623.
25 Barlow, S. M., Morrison, P. J., and Sullivan, F. M., J. Endocr. *66* (1975) 93.
26 Stein, M., Schiavi, R. C., and Camerino, M., Science *191* (1976) 435.
27 Kilbourne, E. D., Smart, K. M., and Porkorny, B. A., Nature *190* (1961) 650.
28 Haynes, B. F., and Fauci, A. S., J. clin. Invest. *61* (1978) 703.
29 Moretta, L., Webb, S. R., Grossi, C. E., Lydyard, P. M., and Cooper, M. D., J. exp. Med. *146* (1977) 184.
30 Hochman, P. S., and Cudkowicz, G., J. Immun. *119* (1977) 2013.
31 Gray, J. G., Pratt, W. B., and Aronow, L., Biochemistry *10* (1971) 227.
32 Lin, Y. L., and Askonas, B. A., J. exp. Med. *154* (1981) 225.
33 Kesson, A. M., Blanden, R. V., and Mullbacher, A., J. gen. Virol. *68* (1987) 2001.
34 Mims, C. A., and Blanden, R. V., Infection Immunity *6* (1972) 695.
35 Oldstone, M. B. A., Blount, P., Southern, P. J., and Lampert, P. W., Nature *321* (1986) 239.
36 McFarland, H. F., Griffin, D. E., and Johnson, R. T., J. exp. Med. *136* (1972) 216.
37 Cole, G. A., and Nathanson, N., Nature *220* (1968) 399.
38 Camenga, D. L., Nathanson, N., and Cole, G. A., J. infect. Dis. *130* (1974) 634.
39 Albrect, P., Curr. Top. Microbiol. Immun. *43* (1968) 44.
40 Johnson, R. T., Viral Infections of the Nervous System, p. 1–81. Raven Press, New York 1982.
41 Johnson, R. T., and Mims, C. A., N. Engl. J. Med. *278* (1968) 84.

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Stress-induced increase in noradrenaline release in the rat hypothalamus assessed by intracranial microdialysis

H. Yokoo, M. Tanaka, T. Tanaka and A. Tsuda

Department of Pharmacology, Kurume University School of Medicine, Kurume 830 (Japan)
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Summary. The hypothalamic microdialysis of conscious rats was used to investigate the effects of immobilization stress (20 min) on extracellular noradrenaline(NA) levels. The stress significantly increased NA levels relative to basal efflux by 106% and this elevation continued for 40 min after release from stress.
Key words. In vivo microdialysis; stress; noradrenaline release; hypothalamus; high performance liquid chromatography; rat.

The activation of central noradrenergic systems by various stressful stimuli has been demonstrated[1], and the marked increase in noradrenaline (NA) turnover has been reported to occur in the hypothalamus of rodents exposed to stress[2, 3]. We have reported that immobilization stress increases NA turnover in various brain regions[4, 5], which show regional differences in terms of both the degree of responsiveness and its time course.

Recently, both the development of sensitive catecholamine assay methods utilizing high performance liquid chromatography (HPLC) and the introduction of the microdialysis technique have permitted the investigation of endogenous catecholamine release from regional brain areas in vivo[6].

In the present study, in order to investigate the time course of endogenous NA release in the hypothalamus in response to immobilization stress, we attempted the direct measurement of extracellular NA levels in the anterior hypothalamus in conscious rats using a microdialysis method.