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Observing a Fictitious Stressful Event: Haematological Changes, Including Circulating Leukocyte Activation

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The aim of this study was to assess the effect of *watching* a psychological stressful event on the activation of leukocytes in healthy human volunteers. Blood samples were obtained from 32 healthy male and female subjects aged between 20 and 26 years before, during and after either watching an 83-minute horror film that none of the subjects had previously seen (The Texas Chainsaw Massacre, 1974) or by sitting quietly in a room (control group). Total differential cell counts, leukocyte activation as measured by the nitroblue tetrazolium (NBT) test, heart rate and blood pressure (BP) measurements were taken at defined time points. There were significant increases in peripheral circulating leukocytes, the number of activated circulating leukocytes, haemoglobin (Hb) concentration and haematocrit (Hct) in response to the stressor. These were accompanied by significant increases in heart rate, systolic and diastolic BP ($P < 0.05$ from baseline). This is the first reported study on the effects of *observing* a psychologically stressful, albeit fictitious event on circulating leukocyte numbers and the state of leukocyte activation as determined by the nitrotetrazolium test.

Keywords: Psychological stress; Leukocytes; Humans; Fictitious event

INTRODUCTION

There is considerable evidence to support the notion that psychological stress may reduce the effectiveness of the immune system, thus increasing the risk of infection or disease (Dhabhar *et al.*, 1996; Kang *et al.*, 1996). Our research suggests that even short-term psychological stressors can produce demonstrable physiological changes in heart rate, blood pressure (BP) and the activation of neutrophils (Ellard *et al.*, 2001). Activated neutrophils release many mediators, which can potentially damage even healthy tissue and organs, so this activation of neutrophils is potentially detrimental to health (Weiss, 1989; Weiss *et al.*, 1989; Boxer and Smolen, 1998). Many biologically active compounds are released by neutrophils when they are activated, these include: cationic proteins, myeloperoxidase, lysozyme, acid hydrolases, lactoferrin (an iron-binding protein), B12-binding protein, cytochrome b and collagenase (Abramson and Wheeler, 1993).

Epidemiological studies have provided data to support the idea that those individuals who are more psychologically stressed are more prone to opportunistic infections (Clover *et al.*, 1989; Galinowski, 1997). For example, stress associated with family dysfunction has been reported to be significantly associated with increased incidence of upper respiratory tract infection and influenza B (Clover *et al.*, 1989). Similarly, an accumulation of stress in elite athletes has been associated with chronic immunosupression and hence, susceptibility to opportunistic infections (for review see Gleeson, 2000). In animal models of stress, the spread of *Candida albicans* (an opportunistic fungal disease) is greater in stressed than non-stressed animals (Rodriguez-Galan *et al.*, 2001).

Acute psychological stressors have also been shown to increase the number of circulating leukocytes, and to significantly affect erythron variables such as haematocrit (Hct), mean cell Hb content and the number of red blood cells (Maes *et al.*, 1998a,b).

Psychological stress comes in many guises. Some individuals deliberately expose themselves to a form of psychological stress, such as watching a horror movie.

To date, there have been no reported studies on the effect such a "passive stressor" has on leukocyte activation. We asked whether passive observation of a stressful

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event, namely witnessing an emotionally disturbing movie, would result in leukocyte mobilisation and activation. The aim of this study was thus to investigate the effect of *observing* a stressful event, albeit fictitious, on leukocyte distribution and activation in otherwise healthy human subjects

METHODS

The Subjects

Local ethical committee approval from Coventry University Ethics Committee and informed consent was obtained before this study commenced, in accordance with the declaration of Helsinki.

Thirty-two subjects (16 males and 16 females), all moderately fit and healthy aged between 20 and 26 years, participated in the study. Equal numbers of males and females were randomly assigned to either the control or experimental group. Exclusion criteria included suffering from psychiatric illness, respiratory or cardiovascular disease, smokers, or the taking of prescription medicine within the previous month.

Design

The experiments were performed in the afternoon according to highly standardised procedures. Subjects were instructed to avoid exercise or alcohol for 48 h before the study and to fast for 2 h before the study began.

All groups sat quietly for 15 min to obtain resting BP, heart rate and resting blood samples. Blood samples were then taken from all subjects 15 min before exposure to either no stress (control conditions) or psychological stress in the form of watching the horror film. This time point was designated − 15 min. Equal numbers of males and females were assigned to each of the two groups. The allocation to either control or experimental group was on a random basis and subjects had no prior knowledge as to which group they would be assigned to.

Sixteen subjects watched the 83-minute horror film that none of the subjects had previously seen (The Texas Chainsaw Massacre, 1974, directed and produced by Tobe Hooper. Everett Collection, Inc).

The remaining 16 subjects acted as a control group. They were instructed to sit quietly for the same length of time (83 min), under similar lighting conditions and were given emotionally non-stimulating material to read if they so wished. This consisted of text books, articles from journals and health information leaflets.

A repeated measures design was implemented with two groups, the experimental group (watching the horror film) and the control group. Blood samples, heart rate and BP measurements were taken 15 min before, just after (85 min) and 30 min after being exposed to either the "stressful event" or to control conditions.

Heart Rate and Blood Pressure Measurements

A heart rate transceiver (Polar, Heart rate monitor) was attached directly to the chest and heart rate was monitored. Participants were seated, asked to make themselves comfortable, close their eyes and breathe orthonasally. This procedure was carried out for 15 min to minimise possible stress levels experienced prior to, or upon arrival, at the laboratory. At the end of this period baseline heart rate was recorded and the first blood sample taken (see sample protocol below). Participants were then instructed to either read quietly or in a separate room to watch the horror film. Upon completion of the task and 30 min afterwards, heart rate was recorded again, and further blood samples taken.

Systemic BP was measured via the brachial artery using an aneroid sphygmomanometer (Accoson, (Surgical) Ltd, London) and stethoscope (Harvard Ltd, Edenbridge, UK)

Blood Samples

A measurement of 6 ml blood samples were drawn from indwelling venous catheters at the time periods specified into 0.06 ml of 0.34 M EDTA (Vacutainer, Becton Dickinson).

Leukocyte Numbers

Total leukocyte counts, leukocyte subset counts (neutrophils, lymphocytes, monocytes), Hb and Hct values were taken from full blood count data that were measured using an automated system (Technicon H2 system). The interassay CV values in the laboratory were $<2\%$ for the leukocyte count and differentials.

Nitrotetrazolium Blue Staining

Blood samples were stained soon after collection. 20 μl aliquots were mixed with an equal quantity of Nitroblue tetrazolium (NBT) (Sigma Diagnostics cat. no. 840-10) diluted at 1 mg/ml with phosphate buffered saline (0.9%, pH 7.4). These samples were placed on coded microscope slides, incubated at 37°C for 10 min, and then air-dried (two samples were prepared for each subject at each timepoint). Once dry the samples were flooded with Accustain Wright Modified stain (0.3%w/v buffered at pH 6.9 in methanol. Batch no. 096 H4372) and rinsed with distilled water. The intra-assay CV for the NBT test was $<5\%$ in our laboratory.

Determining the Percentage of Activated Leukocytes

Using a × 100 oil immersion objective, four areas of each slide were examined with a microscope. The total numbers of dormant and activated neutrophils were counted. Leukocytes were classified to be active if they appeared irregular in shape and diffusely granular with intracytoplasmic formazan deposits. Features such as cell shape,

clump formation, presence of formazan deposits or granular constituents and cell dimensions were recorded.

Data Analyses

Data are expressed as means + standard error of mean (SEM). MANOVA was used to test the main effect for the experimental group (horror film, control), occasion (− 15, 85, 115 min), and interaction effect. A 2-tailed unpaired *t*-test was subsequently used to compare the 2 groups for change at 85 and 115 min from baseline values (assessed at − 15 min).

RESULTS

Haematological variables. The overall changes in haematological variables in the horror film group and the control group are displayed graphically (means with SEM bars) in Fig. 1. Table I shows the relevant MANOVA *p*-values for each of the seven haematological variables. Statistical significance was found for experimental group, occasion and interaction effect.

Table II shows the relevant unpaired 2-tailed *t*-test *p*-values when comparing the changes from baseline between the horror film group ($n = 16$) and the control group ($n = 16$) for each haematological variable. A statistically significant ($p < 0.05$) increase was found at 85 min in the horror film group when compared to the control group for each of the seven variables.

In particular, the percentage of activated leukocytes in the stress group was increased, and this persisted for at least 30 min after watching the horror film. There were also increases, restored to basal within 30 min after watching the horror film, in the leukocyte and lymphocyte counts. There were smaller increases in the monocyte and

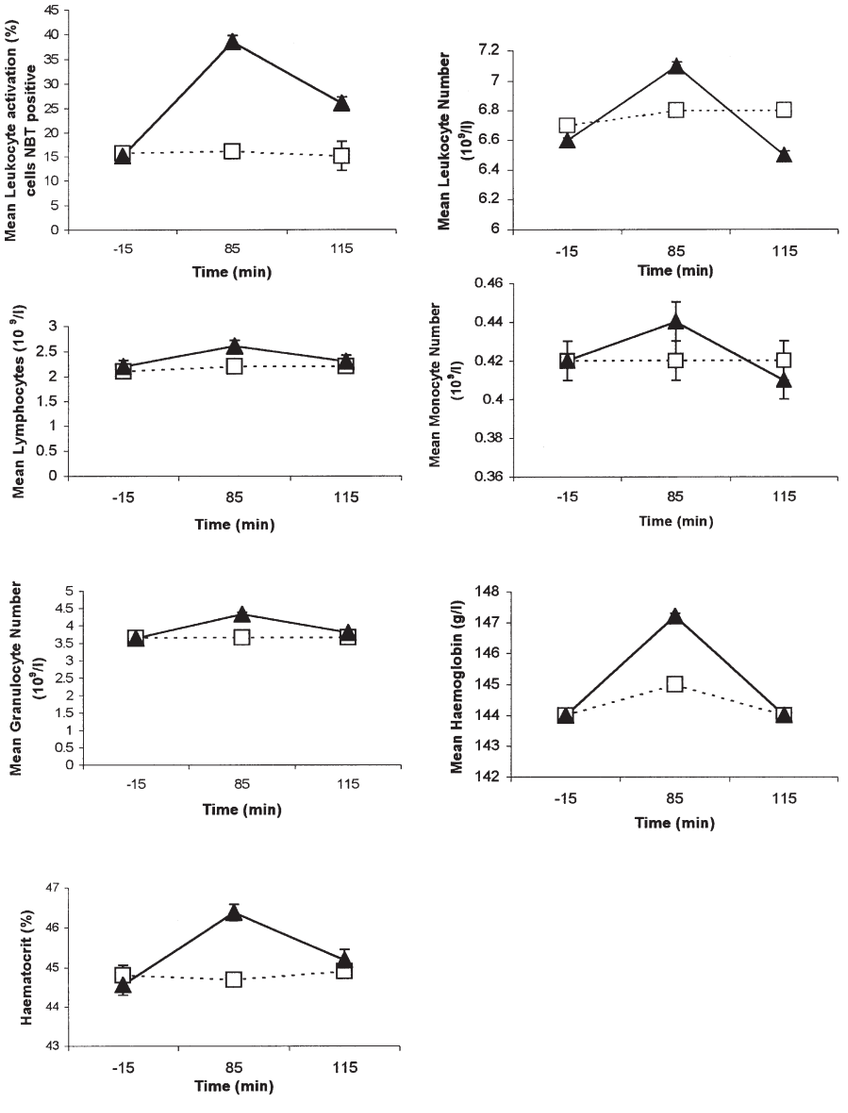

FIGURE 1 Haematological variables: the effect of watching a horror film. Open squares represents mean ± SEM from 16 control subjects. Closed triangles represent mean ± SEM from 16 subjects exposed to the horror film for all graphs. Statistical analyses are in Tables I and II.

TABLE I MANOVA results for 2 × 3 (experimental group × occasion) model

	p-values		
Haematological variable	Exp. Group (between subjects)	Occasion (within subjects)	Exp.Group × Occasion (interaction)
NBT	<0.0001	<0.0001	<0.0001
Leukocytes	<0.0001	<0.0001	<0.0001
Lymphocytes	<0.0001	<0.0001	<0.0001
Monocytes	0.3451	0.0022	0.0466*
Granulocytes	0.0172	<0.0001	<0.0001
Haematocrit	0.1103	<0.0001	<0.0001
Haemoglobin	<0.0001	<0.0001	<0.0001
HR	<0.0001	<0.0001	<0.0001
Systolic BP	<0.0001	<0.0001	<0.0001
Diastolic BP	0.0005	<0.0001	<0.0001

*On application of the Greenhouse–Geisser correction, $p = 0.0696$ (for all other interactions, the Greenhouse–Geisser yielded $p < 0.0001$).

granulocyte counts. Haemoconcentration was indicated in the stress group by increased Hct and Hb concentration (Fig. 1; Tables I and II). These changes were similar in extent, as the percentage increases from basal (4.2 and 2.2%, respectively), to the increases in monocyte and granulocyte counts (4.8 and 1.9%, respectively), suggesting that these changes may be due to haemoconcentration. In contrast, the increases in leukocyte and lymphocyte counts (10.6 and 18.1%, respectively) were several-fold greater than the changes in Hb and Hct values. There were no significant changes with time in the control group.

Heart rate and BP. The average heart rate change of +14.19 bpm for the horror film group represents a 21.6% increase (Table III). Systolic and diastolic BP increased in this group by 10.8 and 16.9%, respectively. The control group showed no such changes. Statistical significance was found for experimental group, occasion and interaction effect (Table I). For the changes in pulse rate and systolic and diastolic BPs a statistically significant ($p < 0.05$) increase was found at 85 min in the horror film group when compared to the control group for each of the three variables (Fig. 2; Table III). There were no significant changes with time in the control group.

DISCUSSION

Watching a horror movie elicits a psychophysiological arousal which is comparable to Canon's fear flight fight defence reaction, the so called "stress response" which involves stimulation of the hypothalamus (Canon, 1932; Folkow, 1982), a change in peripheral resistance (Brod, 1970) and an increase in the release of stress hormones including catecholamines and cortisol (Selye, 1946) and increases in Hct and Hb concentration (Maes *et al.*, 1998a,b).

The increase in the numbers of circulating leukocytes during the horror film stress is comparable to that reported by others. Changes in circulating cell numbers reflects the cell trafficking between reservoir sites including the liver, lungs, spleen, bone marrow and peripheral blood (Cruse and Lewis, 1995). This process is modified by receptors (Ley, 1996) on both the endothelium

TABLE II Summary of haematological values

Haematological variable	−15 min Baseline	85 min Change from baseline	115 min Change from baseline
NBT Control (%)	15.63 (0.98)	0.63 (0.44)	−0.06 (0.30)
Horror group	15.06 (0.92)	23.88 (1.23)*	10.56 (1.24)*
Leukocytes (10^9/l)			
Control	6.7 (0.02)	0.1 (0.02)	0.1 (0.24)
Horror group	6.6 (0.02)	0.7 (0.02)*	0.1 (0.02)
Lymphocytes (10^9/l)			
Control	2.1 (0.12)	0.1 (0.13)	0.1 (0.13)
Horror group	2.2 (0.1))	0.4 (0.12)*	0.1 (0.11)
Monocytes (10^9/l)			
Control	0.42 (0.01)	0.01 (0.01)	0.00 (0.01)
Horror group	0.42 (0.01)	0.02 (0.01)*	−0.01 (0.01)
Granulocytes (10^9/l)			
Control	3.65 (0.08)	0.01 (0.05)	0.01 (0.01)
Horror group	3.63 (0.08)	0.07 (0.06)*	0.15 (0.050)*
Haematocrit (%)			
Control	44.88 (0.26)	−0.19 (0.16)	0.06 (0.06)
Horror group	44.56 (0.27)	1.88 (0.20)*	0.63 (0.26)
Haemoglobin (g/l)			
Control	144 (0.1)	1.0 (0.1)	0.01 (0.1)
Horror group	144 (0.1)	3.2 (0.1)*	0.01 (0.1)

The Satterwaite method (unequal variances) was used to derive all *p*-values.
Values are means with SEM shown in brackets; $n = 16$ per group.
*$P < 0.05$ from control.

TABLE III Heart rate and blood pressure changes

HR (bpm)			
Control	65.00 (0.45)	1.00 (0.63)	0.00 (0.22)
Horror group	65.69 (0.41)	14.19 (0.71)*	−0.63 (0.50
Systolic BP (mmHg)			
Control	126.25 (0.52)	0.44 (0.38)	0.00 (0.18)
Horror group	125.44 (0.44)	13.56 (0.77)*	1.06 (0.38)
Diastolic BP (mmHg)			
Control	69.69 (0.77)	0.94 (0.35)	0.19 (0.60)
Horror group	70.31 (0.66)	11.88 (0.55)*	−3.31 (0.96)*

The Satterwaite method (unequal variances) was used to derive all p-values.
Values are means with SEM shown in brackets; $n = 16$ per group.
*$P < 0.05$ from control.

(P-selectin; Intracellular Adhesion Molecule-1;. Vascular cell Adhesion Molecule-1) and leukocytes (L-selectin; integrins and PSGL-1 P-Selectin Glycoprotein Ligand-1). Modification of the receptors on either the endothelial cells or leukocytes can also dramatically alter the number of adherent (and thus the number of free flowing) leukocytes (Ley, 1996; Ley, 1997).

PSGL-1 is constitutively expressed on all lymphocytes, monocytes, eosinophils and neutrophils (Ley, 1997). PSGL-1 has a glycosylation pattern enabling it to bind to endothelial P-selectin (Ley, 1997). This interaction results in the margination of the leukocytes, which is the process by which leukocytes exit the central blood stream, and initiate mechanical contact with the endothelial cells (Cruse and Lewis, 1995).

The margination process is enhanced in particular sized vessels by the aggregation of erythrocytes, which tend to occupy the centre of microvessels and thus promote margination (Firrell and Lipowsky, 1989). The increase in Hct and Hb concentration observed in this and previous studies (Maes *et al.*, 1998a,b) could thus selectively promote margination in some vessels. Previous studies have demonstrated that margination of leukocytes is not a uniform process, and occurs in particular sized vessels within the microcirculation (Mian and Marshall, 1993). In larger sized vessels it is possible that the shear stress of flowing blood might be sufficient to dislodge marginated leukocytes, thus increasing the numbers of free-flowing leukocytes. The changes in shear stress likely to have been brought about by the increased haemoconcentration may also serve as a trigger mechanism for leukocyte activation (Schmid-Schonbeim *et al.*, 2001).

It has been recognised for some time that physiological stressors such as exercise induce leucocytosis from marginal pools (Shepard and Shek, 1996; Gleeson *et al.*, 1998). Current literature indicates that exposure to hostile conditions or other psychological stressors initiates the secretion of several hormones, including cortisol, catecholamines, prolactin, oxytocin and renin (Toft *et al.*, 1994; Van de Kar and Blair, 1999), any of which could alter adhesion receptors on circulating leukocytes and thus contribute to their altered distribution. It is believed that stimuli such as adrenaline are able to disrupt this process (Iversen *et al.*, 1994) and increase the circulating numbers of leukocytes. Recent studies by Maes *et al.* (1999) have revealed that an increase in the levels of pro-inflammatory cytokines, such as interleukin-6 and tumour necrosis factor, result in the demargination of some leukocytes. It is thus possible that the stress -induced production of adrenaline and cytokines could orchestrate the increased numbers of leukocytes within the general circulation.

However, non-physical stressors have now also been shown to influence the number and distribution of leukocytes in the blood. Kang *et al.* (1996) and Dhabhar *et al.* (1996) reported that the mental stress of academic examinations stimulated increases in the number and distribution of leukocytes. These changes were found to be both rapid and reversible.

NBT reduction was selected as a measure of cell activation to minimise *in vitro* manipulation of fresh

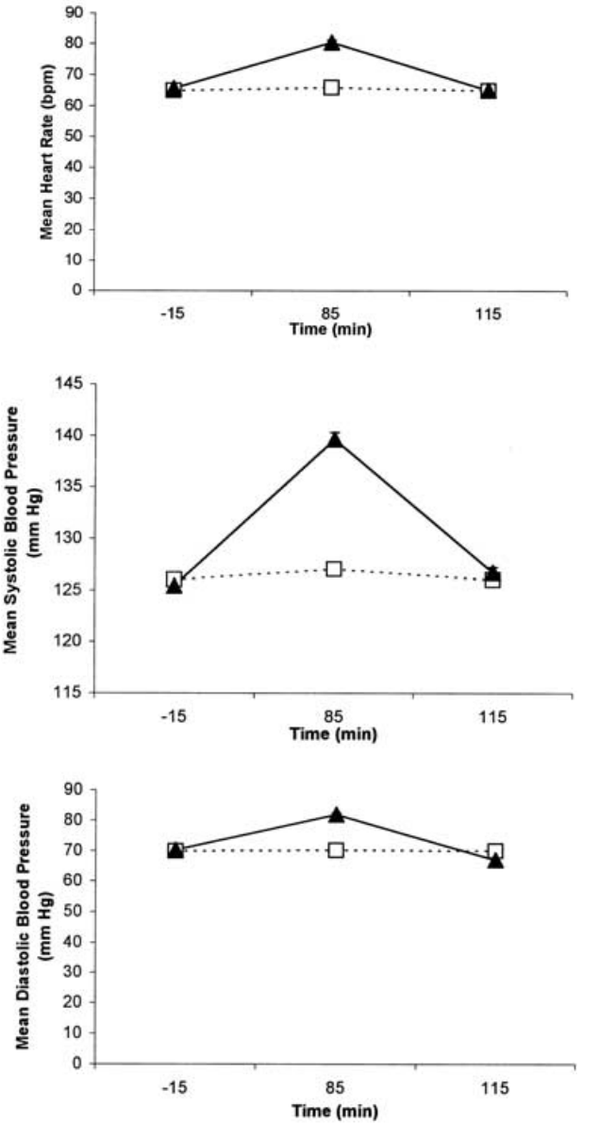

FIGURE 2 Heart rate and arterial BP: The effect of watching a horror film. Open squares represents mean ± SEM from 16 control subjects. Closed triangles represent mean ± SEM from 16 subjects exposed to the horror film for all graphs. Statistical analyses are in Tables I and III.

blood, and to reflect as far as possible the *in vivo* condition of the cells. Recent studies have demonstrated that the NBT reduction assay is a reliable measure of the activation of neutrophils in whole blood (Takase *et al.*, 1999; Delano *et al.*, 1997). Wikstrom *et al.* (1996) demonstrated a highly significant linear relationship between the % NBT -positive cells and chemiluminescence measurements of the same cell suspensions with and without chemical stimulation, supporting the idea that the NBT-test accurately measures oxidative metabolism. Thus the significant increase in the number of NBT leukocytes after watching the horror film reflects an increase in the numbers of activated leukocytes. Activation of leukocytes has been reported by Kang *et al.* (1996), who found that superoxidase production in neutrophils increased in those undertaking examinations.

Our study is the first to support the notion that even watching a stressful event may be sufficient to stimulate leukocytosis and activation that is rapidly reversible. Whether this reflects an activation process *per se* or whether this occurs as a result of an increased trafficking of activated leukocytes, remains elusive.

In a broader context, the pathophysiological relevance of the changes observed need to be explored. If the human mind cannot dissociate observation of fictional stressful situations from personal psychological experience, then the results have implications for anyone witnessing a stressful event. Witnessing a stressful event could well be sufficient to alter the number and activation state of circulating leukocytes. Indeed the percentage of activated leukocytes remained high even though many of the other variables such as the number of circulating leukocytes and Hct and Hb concentration had returned to basal (pre-stress) values. In this state of activation, leukocytes are primed and ready for action. If, however, the leukocytes actually release the contents of their granules, then there will be a period of time, a "window of opportunity", in which they will not be able to respond to opportunistic infections, having already degranulated. Such leukocytes would be unable to respond to opportunistic infections thus rendering the host more susceptible to disease, as well as to potential tissue damage from a host of proteolytic enzymes and oxygen free radicals.

Unlike other cardiovascular and immune measurements, leukocyte activation was sustained after the stress exposure, suggesting that this condition of the leukocytes is not rapidly reversible. The potential long-term effects of repeatedly watching such horror films remain unknown. It is possible that repeated stressors could well affect resting values of the measured parameters.

In an era, where television and other electronic media rapidly transmit real and fictitious events into our homes, the effects of exposure to such stressors deserves a serious and systematic evaluation. We conclude that witnessing a stressful event may have serious physiological consequences for the health of the observer. With sufficient exposure to psychological stress is it possible that an observer actually becomes a victim by proxy? This possibility needs to be investigated.

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