A ternary model of decompression sickness in the rat

by

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Abstract

Introduction: Decompression sickness (DCS) in the rat is most commonly modelled as a binary outcome. The present study aimed to develop a ternary model of predicting probability of DCS in the rat, (as no-DCS, survivable-DCS or death), based upon the compression/decompression profile and physiological characteristics of each rat.

Methods: A literature search identified dive profiles with outcomes no-DCS, survivable-DCS or death by DCS. Inclusion criteria were that at least one rat was represented in each DCS status, not treated with drugs or simulated ascent to altitude, that strain, sex, breathing gases and compression/decompression profile were described, and that weight was reported. A dataset was compiled (n=1602 rats) from 15 studies using 22 dive profiles and two strains of both sexes. Inert gas pressures in five compartments were estimated. Model-fit of the calibration dataset, using ordinal logistic regression, was optimised by maximum log likelihood and likelihood ratio test. Two validation datasets (one interpolation, one extrapolation) assessed model robustness.

Results:

$$\Pr[DCS = j \mid x_i] = \underline{\alpha} + 0.015Weight_i + 1.435Female_i - 42.956Max.1_i + 43.350Max.1b_i + 2.166Bubble.3_i$$

Where α_1=-25.483, α_2=-26.838

In the interpolation dataset the model predicted 10/15 cases of nDCS, 3/3 sDCS and 2/2 dDCS, totalling 15/20 (75% accuracy) and 18.5/20 (92.5%) were within 95% confidence intervals. Mean weight in the extrapolation dataset was more than 2 SD outside of the calibration dataset and the probability of each outcome was not predictable.

Discussion: This model is reliable for the prediction of DCS status providing the dive profile and rat characteristics are within the range of parameters used to optimise the model. The addition of data with a wider range of parameters should improve the applicability of the model.

Keywords: Decompression illness, ordinal logistic regression, modelling, marginal decompression sickness, animal model, trinary outcome

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Introduction

Animal models offer an alternative to human studies into decompression sickness (DCS) that is both ethically preferable for speculative research and logistically convenient. Prawns, mice, rabbits, dogs, goats, pigs and primates have all contributed to mankind's understanding of DCS but the leading role in animal model research surely belongs to the laboratory rat, *Rattus norvegicus*. Pressure exposures designed to elicit DCS in only a proportion of rats vary in depth, time at maximum exposure, breathing gas, rates of compression/decompression and other parameters. Treatments and/or risk factors are then typically evaluated by the degree of difference in the proportion of animals that are diagnosed with DCS following decompression.[1]

DCS in the rat has been variously defined and diagnostic criteria include survival time,[2-4] observable signs such as walking difficulties,[3, 5-14] paralysis,[5-19] rolling in a rotating cage,[5-9, 12, 13, 15, 16, 20] twitching/convulsions[5-9, 12, 13, 15, 16] and/or respiratory distress[5-7, 9-11, 13, 14, 17-19]. Objective measures have been proposed, in particular observable or audible bubble grades.[10, 21, 22] Only rarely have objective measures been correlated with subjective observer agreement. Recently a promising grip-score test was found significantly associated (p=0.004) with observable signs of what was assumed to have been DCS.[23] Unexpectedly, based upon the correlation between loss of grip strength and perceived DCS, Buzzacott et al discovered the post-decompression probability of any asymptomatic rat having DCS was 0.5. The precise diagnosis of DCS in the rat, therefore, remains a desirable goal.

In almost all studies to date DCS in the rat has been modelled as the probability of either no-DCS vs. DCS,[9, 12, 15, 16, 24, 25] or as Dead vs. Alive[4, 11]. Occasionally both models will be sequentially used in the same study but without delineating the relative probabilities of each DCS status.[5, 26] To our knowledge, only one study has used ordinal logistic regression for ternary DCS outcomes in the rat, for an assessment of the effects of ascent rate and post-dive exercise.[27] In this study Pollard and colleagues used ordinal logistic regression to model the probability *p* of a DCS outcome state *j* (either no DCS, survivable DCS or death), given *i* independent covariates $x_{1:n}$ with respective coefficients $\beta_{1:n}$, as

$$Ln\left(\frac{p_j}{1-p_j}\right) = \underline{\alpha} + \sum_{i}^{n} \beta_i x_i \qquad (1)$$

where $\underline{\alpha} = [\alpha_1, \alpha_2, \ldots \alpha_k]$ is a vector of intercepts (one less than the number of outcome states). For k+1 states, the probability of the i^{th} observation being in state j is given in Equation 2.

$$\Pr[DCS{=}j|x_i] = \begin{cases} \Pr[DCS \le 1|x_i] & j = 1 \\ \Pr[DCS \le j|x_i] - \Pr[DCS \le j-1|x_i] & 1 < j \le k \\ 1 - \Pr[DCS \le k] & j = k+1 \end{cases} \qquad (2)$$

The present study aimed to develop a ternary model of predicting the probability of DCS in the rat, (as either no DCS, survivable DCS or death), based upon compression/decompression

profile-dependent inert gas compartment pressure estimates, after adjustment for sex, weight and strain.

Methods

An electronic literature search identified protocols with compression/decompression profiles that elicited a predictable proportion of DCS greater than 0 but less than 100%. From these, studies classifying decompression outcomes as no-DCS (nDCS), survivable-DCS (sDCS) or death by DCS (dDCS) were identified. The inclusion criteria for the rats in each study were that at least one rat was represented in each DCS classification post-decompression to 1 ATA, that the rats were not treated (or pre-treated) with experimental drugs or exercise (only control rats were included in our dataset), that the strain, sex, breathing gases (only oxygen:nitrogen combinations) and compression/decompression profile were described, and that either individual weights or the group mean with relatively small standard deviation (<15% of the mean) were reported. Where only one of these parameters was unclear in the published paper then the original authors were contacted with a request to clarify the missing detail. Only 100% complete data was accepted into the dataset. As soon as the dataset contained in excess of 1600 rats then further inputting was curtailed. By this stage the dataset had been compiled from 15 studies[2-4, 7, 10, 13, 17, 18, 20, 22, 28-32] using 22 different dive profiles and two strains of rat, Sprague-Dawley (n=1421, 89%) and Wistar (n=181, 11%).

Diagnostic criteria for DCS classification was either explicitly stated in each paper (i.e. based on observed respiratory distress or motor ataxia) or else implied by gas emboli score.[4, 22] From the description of each profile, ambient and gas partial pressures in msw at 10 sec intervals or less were calculated in MS Excel. Using the R package SCUBA, stepwise inert gas pressures (in ATA) in 17 Bühlmann compartments (ZH-L16A) were estimated from the MS Excel profiles.[33, 34] As rats are thought to saturate in less than 90 mins,[6, 26, 35] only compartments 1-4 (including 1b) with nitrogen half-times of 4.0, 5.0, 8.0, 12.5 and 18.5 mins respectively were included in the initial model,[34] shown in Equation 3. Longer total saturation times have been proposed but are the exception.[36] From the estimated compartment inert gas pressures two parameters were estimated. For each compartment the maximum positive difference between compartment inert gas pressure and inspired inert gas pressure (in ATA) during ascent ($Max_{1\text{-}4}$: a measure of positive pressure gradient, for off-gassing) and maximum positive difference between compartment inert gas pressure and ambient pressure (in ATA) during ascent ($Bubble_{1\text{-}4}$: a measure of bubble production capacity). Model optimisation is described below, in Analysis.

$$\begin{aligned} Logit\left[\Pr(DCS = j \mid x_i)\right] = {} & \alpha_j + \beta_1 Weight_i + \beta_2 Strain_i + \beta_3 Sex_i + \beta_4 Age_i + \beta_5 Dive_i + \\ & \beta_6 Exercise_i + \beta_7 Max1_i + \beta_8 Max1b_i + \beta_9 Max2_i + \beta_{10} Max3_i + \beta_{11} Max4_i + \beta_{12} Bubble1_i + \\ & \beta_{13} Bubble1b_i + \beta_{14} Bubble2_i + \beta_{15} Bubble3_i + \beta_{16} Bubble4_i \end{aligned} \quad (3)$$

where DCS was nDCS=0, sDCS=1 and dDCS=2. Weight=the weight in grams, Strain was either Sprague-Dawley (0) or Wistar (1), Sex was 0 for male and 1 for female, age was in

whole weeks, Dive was the stratification variable for which particular compression/decompression profile each rat underwent, Exercise was if each rat exercised in a rotating wheel either during or after the dive, where no exercise=0 and with exercise=1. The final model was optimised by logistic regression and backwards elimination of least significant parameters. At n=1602 rats in the calibration dataset, there was an initial mean of no less than 27 rats per parameter in each of the three outcome status', nearly triple the recommended minimum.[37]

To validate the resultant model for interpolation two control groups (from previous experiments) of 10 male (age 11 wks, wt 401±18) and 10 female (age 14 wks, wt 266±22) rats were combined. These 20 rats had been compressed and decompressed according to the protocol (Figure 1) described by Eftedal, *vide infra.*[22] This profile, but not these rats, was included in the calibration dataset. To validate the resultant model for extrapolation 119 control rats from four previous experiments (109 male and 10 female) were combined into a single dataset, including 20 Wistar (wt 384±15) and 99 Sprague-Dawley (wt 428±60), age 10-13 wks.

All rats in the validation datasets were obtained from Janvier SAS (Le Genest St Isle, France) at age 10 weeks. The rats were housed for at least one week in the University vivarium in standard conditions, (mean temperature 21.2°C +/- 0.2 SD, relative humidity 27% +/- 16% SD, 12 hour light:dark cycle), during which they had access to rat chow and water *ad libitum*. The rats were weighed on the day of diving and then compressed in a 170-litre Comex hyperbaric chamber in groups of up to seven at a time. All dives commenced in the morning after 8am.

For the interpolation profile, compression with air occurred at the rate of 2 ATA.min^{-1} to a pressure of 7 ATA (60 msw) and maintained for 45 mins. At the end of the exposure period these rats were decompressed linearly to the surface at a rate of -0.5 ATA.min^{-1}. Total duration of the hyperbaric exposure was 60 mins. For the extrapolation profile, compression using air to 10 ATA (90 msw) occurred at the rate of 1 ATA.min^{-1}. Maximum pressure was maintained for 45 mins followed by decompression at -1 ATA.min^{-1} to 2 ATA (10 msw). Decompression was thereafter staged with five mins at 2 ATA, five mins at 1.60 ATA (6 msw) and 10 mins at 1.3 ATA (3 msw)(Figure 1). Total hyperbaric exposure for the extrapolation dataset was 83 mins. Both these protocols have been shown to produce DCS signs in a predictable proportion of male and female Sprague-Dawley and Wistar rats aged 10-13 weeks.[1, 23, 38]

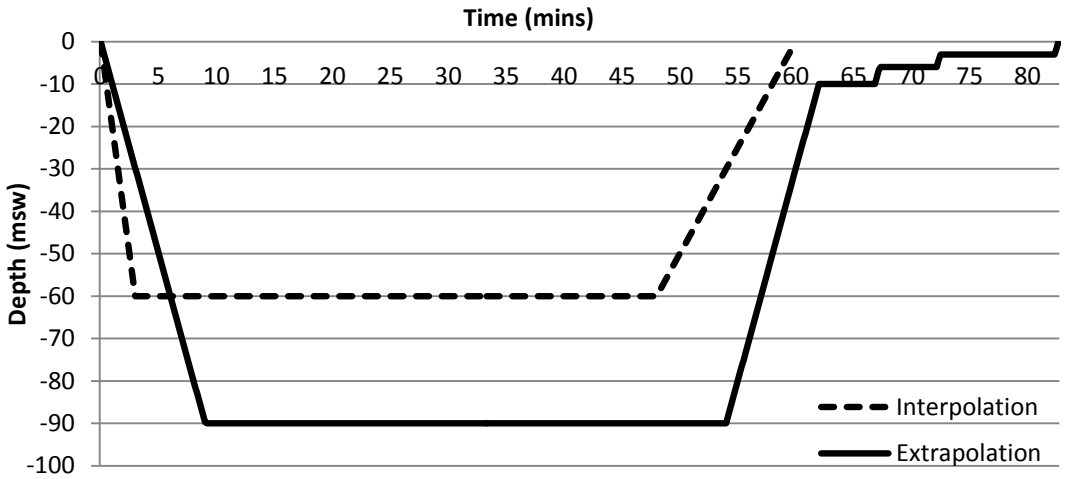

Figure 1: Time-pressure profiles of the interpolation and extrapolation datasets

Following decompression in either profile the rats were quickly removed from the chamber and observed for signs of DCS for one hour. The scale used was No observable DCS (nDCS)=0, respiratory distress or paralysis (sDCS)=1 and death within one hour (dDCS)=2. Two observers agreed the diagnosis in each case. Time of death was recorded as occurring at 0 minutes if observed when the chamber was opened or at the time since surfacing that death occurred in all other cases. Survival was censored at the end of the observation period at 60 minutes, a common length of time in rat DCS studies.[11, 22, 31]

This research was approved by the French Ministry of Agriculture and the Universite de Bretagne Occidentale animal research ethic committee (R-2011-FG-01).

Analysis

Data were analysed using SAS ver 9.3 (SAS, Cary, North Carolina). Ordinal (ternary) logistic regression model fit of the calibration dataset was optimised through backwards elimination of least significant parameters by the maximum log likelihood and likelihood ratio test, which is appropriate for nested datasets such as when one parameter at a time is removed from a dataset containing no missing data. Significance was accepted at $p<0.05$. Using the resultant model, probability of DCS was then predicted for each rat in both validation datasets, by the back transformation of Equation 1 using Equations 4 and 5. From the mean probability of DCS (by outcome status) a total number of rats in each outcome status was predicted with confidence intervals.

$$\text{If } \mathrm{Ln}[p/(1-p)] = B \quad (4)$$

$$\text{Then } p = e^{B}/(1+e^{B}) = 1/(1+e^{-B}) \quad (5)$$

Results

Following the elimination of non-significant parameters, the resultant model is shown in Equation 6.

$$Logit\left[\Pr(DCS = j \mid x_i)\right] = \underline{\alpha} + 0.015Weight_i + 1.435Female_i - 42.956Max.1_i + 43.350Max.1b_i + 2.166Bubble..3_i \quad (6)$$

Where α_1=-25.483, α_2=-26.838

Adjusted odds ratios with confidence limits and p-values are given for the retained parameters in Table 1.

Table 1: Adjusted odds ratios with confidence intervals and p-values for the parameters retained in the final model

		Odds Ratio	95% CI	P-value
Weight	(g)	1.02	1.01, 1.02	<0.0001
Female		17.6	6.5, 47.7	<0.0001
Max1	(ATA)	<0.01	<0.01, 0.04	0.03
Max1b	(ATA)	>999	35.0, >999	0.03
Bubble3	(ATA)	8.73	5.24, 14.5	<0.0001

The characteristics of the calibration and validation datasets are presented in Table 2, by DCS outcome and overall. Probability of nDCS, sDCS or dDCS were calculated for each rat in the validation datasets using equation 5. The mean predicted nDCS, sDCS and dDCS are also shown in Table 2, with 95% confidence intervals..

Table 2: Characteristics of rats in the calibration and validation datasets by DCS outcome and overall

Calibration		No DCS (n=699, 44%)	Severe DCS (n=438, 27%)	Death by DCS (n=465, 29%)	Overall (n=1602, 100%)
Weight	g (SD)	256 (±65)	257 (±62)	298 (±53)	268 (±63)
Female	n (%)	60 (9)	25 (6)	62 (13)	147 (9)
Interpolation		No DCS (n=15, 75%)	Severe DCS (n=3, 15%)	Death by DCS (n=2, 10%)	Overall (n=20, 100%)
Weight	g (SD)	330±77	387±6	277±28	333±72
Female	n (%)	8 (53)	0 (0)	2 (100)	10 (50)
Predicted	n	9.9	5.8	4.4	20
(95% CI)		(5.2-13.5)	(4.3-6.5)	(2.2-8.7)	-
Extrapolation		No DCS (n=38, 32%)	Severe DCS (n=17, 14%)	Death by DCS (n=64, 54%)	Overall (n=119, 100%)
Weight	g (SD)	402±67	420±66	431±47	420±58
Female	n (%)	6 (16)	2 (11)	2 (3)	10 (8)
Predicted	n	119	0	0	119
(95% CI)		(119-119)	(0-0)	(0-0)	-

The predicted outcomes in Table 2 indicate that for the interpolation validation dataset the model predicted 9.9/15 cases of nDCS, 3/3 sDCS and 2/2 dDCS, totalling 14.9/20 (75% accuracy) and 13.5/15 nDCS, 3/3 sDCS and 2/2 dDCS (18.5/20, 92.5%) were within 95% confidence intervals. The model over-predicted male DCS. The extrapolation dataset did not allow prediction of DCS (Table 2) and all rats were predicted in the nDCS outcome. Table 3 compares the parameter values between calibration and validation datasets. Weight is clearly different between datasets. Mean values in the calibration dataset for Max1 and Max1b were 3.8 and 4.1 ATA respectively (Table 3), with coefficients in the final model of -42.956 and +43.350 respectively. Compartment 1 had a half-time of 4.0 mins and compartment 1b 5.0 mins, therefore following any period of stable compression longer than 30 mins then both compartments would commence decompression at least 98.4% (in effect fully) saturated. Combining mean values for Max1 and Max1b with their respective coefficients [-42.956(3.8) + 43.350(4.1)] yields a total contribution towards the value of the logit of 14.5. If the ascent from any pressure while breathing any particular gas mixture is faster than that needed to result in these mean values for Max1 and Max1b then compartment 1b will retain even more inert gas than compartment 1 and, therefore, the contribution to the logit would increase, thereby increasing the probability of DCS. Thus, the closeness of both the half-times and the model coefficients for compartments 1 and 1b in our model account for the effect of rate of ascent upon the probability of DCS.

Table 3: Characteristics of the calibration and validation datasets; mean (SD)

		Calibration (n=1602)	Interpolation (n=20)	Extrapolation (n=119)
Mean weight	g	268 (±63)	333±72	420±58
Max depth	msw	84 (±15)	60	90
Bottom time	mins	64 (±13)	50	45
Max1	ATA	3.8 (±0.8)	2.0	3.4
Max1b	ATA	4.1 (±0.8)	2.3	3.8
Bubble3	ATA	4.5 (±0.6)	3.1	4.4

Discussion

Binary likelihood functions have underpinned DCS research for half a century yet today improved computing power and advanced statistical analysis packages have made logistic regression and likelihood ratio tests for ordinal polychotomous outcome models more readily available. In this study we have shown that published data exists in sufficient extent to compile calibration datasets of a size comparable with those used in human studies.[39, 40]

Weight is a well-established risk factor for DCS in rats.[6, 26, 35, 41] That it remained throughout elimination speaks for the validity of the resultant model although in this case weight was treated linearly. Further research will determine if a curvilinear transformation will improve model-fit, as suggested by Lillo *et al.*[5, 35] To our knowledge however, this is only the second study to find that female sex is a risk factor for DCS in rats,[38] and the significant effect of sex was independent of weight (there was no interaction between weight and sex). The age of

our female rats in the calibration dataset was at the time they reached sexual maturity. A study on humans investigating the influence of sex on the outcome of altitude DCS did not find significant differences.[42] However women using hormonal contraception showed significantly greater susceptibility to DCS than those not using hormonal contraception during the latter two weeks of the menstrual cycle, implicating the hormonal system's influence.

Max1 and Max1b differ to Bubble1 and Bubble1b in that they focus on the off-gassing diffusion rate in well-perfused tissues (half-times of 4.0 and 5.0 respectively). They are the fastest tissues to off-gas during ascent. Max1 and Max1b are close to each other in effect size, but their interaction was not significant, suggesting their inclusion as separate parameters accounts for the rate of ascent, which is a known risk factor for DCS. The difference between them increases with ascent rate which, thus, increases the probability of DCS. This is well known in diving while the precise effect diffusion rate exerts upon cell membrane integrity remains the focus of some experimental research effort in our laboratory. Early results suggest that, in future improvements to the rat model described here, Max1 and Max1b may be replaced with alternate related parameters, for example inspired oxygen partial pressures. Both their ORs and P-values (Table 1) render Max1 and Max1b tentative in our current model.

The Bubble parameters were estimated by subtracting the ambient pressure at any time during ascent from the estimated pressure in each compartment to yield a raw supersaturation pressure in ATA. That Bubble3 was also significant, given that compartment 3's half-time is 12.5 mins, suggests that compartments in the rat that do not off-gas so swiftly are more likely to produce bubbles. Once again, this is logical and also neatly in keeping with previous research which identified the time for saturation in the rat as one hour.[6] In a compartment with 12.5 mins halftime, 98.4% saturation would occur in 75 mins. Compartment 4, with a halftime of 18.5 mins, would be 98.4% saturated after 111 mins and Bubble4 was eliminated from the model as not significant. Future research will utilise a custom vector of compartment halftimes from 1.0 mins to 18.0 mins in 1 min increments. No doubt this will further improve model-fit.

Sprague-Dawley and Wistar were not significantly different to each other in their resistance to DCS, in either the calibration or extrapolation validation datasets. Furthermore, we experimentally confirmed that DCS incidence in this compression/decompression profile elicited similar incidence of DCS between Wistar and Sprague-Dawely.[23] This should be reassuring to the scientific community who rely on previous research utilising either one strain or the other. That exercise was not significant may be explained by the inclusion criteria that at least one rat must be represented in each outcome state. Accordingly, studies in which the rats exercised used compression/decompression profiles calibrated to produce a proportion of DCS in each category, often empirically. Future research might more specifically investigate models that include exercise, compared with those that do not, to elucidate more precisely the effect of exercise during DCS research involving rats. Exercise may affect tissues with different half-times to protocols with no exercise but this has not yet been shown and would be of interest. No doubt the timing of exercise is also critical as

during maximum compression exercise would increase inert gas uptake and during decompression exercise would increase inert gas washout. This may be another reason Exercise was not found to be significant, because we did not delineate between pre- and post-decompression and hence these opposites cancelled each other out. That DCS differs between the sexes confounds much previous research on exercise and DCS. Appropriate weighting of survivable DCS also requires further work to optimise both maximum log likelihood and the R^2, and exercise may well play a role in this. If sDCS is eventually optimally weighted anywhere between 0.0 (nDCS) and 2.0 (dDCS) then the superiority of ternary DCS classification over either typical binary model will be demonstrated.

As with any meta-analysis the protocols and classification differences between experiments included in this study will have introduced a bias that could prove significant. Including a stratification variable for compression/decompression profile (Dive) somewhat adjusted for that bias, though probably not completely. The number of studies and the size of the calibration dataset is however a potential advantage in the face of this. Future research will calibrate models with even larger datasets containing a wider range of both parameters and parameter values. With an R^2 of 0.18 this model has plenty of room for improvement, confirmed by the extrapolation dataset, and considerably increasing the size of the calibration dataset is a current priority.

Table 3 indicates that the extrapolation profile had compartment pressure parameters that were closer to mean values for the calibration dataset than those of the interpolation profile. All else being equal it is clear the rats in this extrapolation dataset had a mean weight more than two standard deviations heavier than the mean weight of the animals in the calibration dataset. This may explain, at least in part, the inability of the model to predict DCS in the extrapolation dataset. Nonetheless, significance of the independent variables Weight, Sex and Bubble3 ($p<0.0001$) suggest their effect upon the risk of DCS is far from negligible. That the model predicted 75% of observer diagnoses in the interpolation validation dataset (92.5% within 95% CI) also demonstrates a solid foundation upon which to build improved goodness-of-fit. The chi-square test for the proportional odds assumption was significant suggesting that the null hypothesis of unequal independent parameter coefficients may be true although the SAS handbook does suggest that the null is rejected more often than it should, particularly with large datasets containing many variables, as was the case in this study. To accept the null hypothesis in this study would imply that death was by a cause other than DCS, or that diagnosed DCS was not associated with those factors in our model, (which have now been experimentally confirmed). Again, an appropriate weighting for sDCS may have an appreciable effect upon this test. Overall, the relationship between DCS and weight, sex and strain have all been experimentally confirmed in our laboratory,[23, 38] and the relationship between DCS and Max1, Max1b and Bubble3 are in accord with what is known of DCS, namely that ascent rate and supersaturation are key factors, and that saturation in the rat occurs at around 60-90 mins.

Ternary classification of DCS could potentially add power to modelling research and continued development in predictive accuracy is leading towards to the identification of associated parameters which, in turn, may assist mankind identify potential mechanisms of

this arcane disease. Our model is reliable for the prediction of DCS status providing the dive profile and rat characteristics are within the range of parameters used to optimise the model. The addition of further profiles and rats of wider physiological variety will likely improve the robustness of the model.

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