

Differences in response to anaesthetics and analgesics between inbred rat strains

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Summary

Differences in response to analgesic and anaesthetic drugs can partly be attributed to variations in the genetic background of experimental animals. This study was carried out to determine differences in the response of inbred rat strains to a selection of analgesics and drugs used in anaesthetic protocols. A cross between the most contrasting strains can then be phenotyped in future studies in order to localize quantitative trait loci (QTLs) involved in analgesic/anaesthetic drug sensitivity. Eight inbred strains ($n = 6$ rats/strain) were selected for the study: the pigmented ACI, BN and COP strains and the albino F344, LEW, SHR, WAG and WKY strains. Each rat was injected intravenously with two analgesics (buprenorphine 0.05 mg/kg and nalbuphine 1 mg/kg) and three drugs used in anaesthetic protocols (propofol 25 mg/kg, medetomidine 50 µg/kg and ketamine 10 mg/kg), respectively, using a crossover design. Analgesic responses were assessed using an analgesiometric procedure. The sleep time of the rat and, where applicable, the interval between injection and loss of righting reflex were used to determine the anaesthetic response. Six out of eight strains responded significantly different from each other to the analgesic effect of buprenorphine with the ACI strain as hyper-responder. The tail withdrawal latency at 55°C of the F344 and WKY rats using buprenorphine was not significantly different from baseline tail withdrawal latencies. In this study, all strains were non-responsive to the analgesic effects of nalbuphine. The response to all three drugs used in anaesthetic protocols differed significantly among the strains. The F344 and BN strains were relatively resistant to the sedative effects of medetomidine. Use of ketamine was abandoned in the ACI and BN strains when the first two animals of both strains died soon after induction. With all three drugs the sleep time of albino rats was significantly longer compared with that of the pigmented ones. We conclude that the results from this study can be used in future studies where QTLs for the sensitivity to anaesthetic/analgesic drugs are localized.

Keywords Strain differences; rat; buprenorphine; nalbuphine; propofol; medetomidine; ketamine; analgesiometry; sleep time

Experimental procedures in animals often require the use of anaesthetics and analgesics to minimize discomfort. In small rodents, they are usually administered as a

single intramuscular, subcutaneous or intraperitoneal injection, which leaves no opportunity to adjust the dose (Flecknell 1996). It is common knowledge that the animal's response to these drugs can show great inter-individual variation. Moreover, it has been well established that this variation

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can partly be attributed to differences in genetic background. Previous studies have demonstrated significant strain differences in the response to propofol, ketamine and medetomidine in the rabbit (Avsaroglu *et al.* 2003) and to pentobarbital in the rat (Collins & Lott 1968, Shearer *et al.* 1973, Vieregge *et al.* 1987). In one of the rat studies, the lethal dose (LD₅₀) for sodium pentobarbital administered intraperitoneally was significantly lower for several albino strains as compared with pigmented strains of rats (Shearer *et al.* 1973), suggesting that susceptibility for sodium pentobarbital is genetically linked to the albino (tyrosinase) locus on rat chromosome 1 (RNO1).

Differences in the antinociceptive effect of analgesics have been demonstrated among some rat inbred strains (Cook *et al.* 2000, Barrett *et al.* 2002, Turner *et al.* 2003).

The present study was designed in accordance with our previous study using inbred rabbit strains (Avsaroglu *et al.* 2003). In both studies, a common objective is shared: phenotyping inbred strains for the response to anaesthetic/analgesic drugs to ultimately localize quantitative trait loci (QTLs) for drug sensitivity using phenotypic information from a cross between contrasting strains. It has been shown that the combined use of low efficacy opioids (i.e. opioids that require activation of a large fraction of the receptor pool to produce an effect) and high-intensive nociceptive stimuli evoke maximal activation of the opioid system, and thus increase the likelihood of finding strain and sex differences (Cook *et al.* 2000, Barrett *et al.* 2002). The partial agonists buprenorphine and nalbuphine, being typical representatives of low efficacy opioids, were therefore selected for our experiment. For obvious reasons, anaesthetic combinations could not be used for this study. Instead, specific drugs used in anaesthetic protocols for laboratory animals were selected based on receptor activity specificity (gamma-aminobutyric acid [GABA], N-methyl-D-aspartic acid [NMDA] and alpha2A). All substances were also selected because of the possibility of intravenous injection to rule out differences in bioavailability

(Pugh 1991). The choice of inbred rat strain was based on three criteria: (i) phylogenetically distant strains (Canzian 1997); (ii) extensively used strains (Hedrich 1990); (iii) inclusion of both albino and pigmented rat strains.

Materials and methods

Animals

Forty-eight male rats of eight different inbred strains ($n = 6/\text{strain}$) were used for the study. The pigmented strains were ACI/SegHsd, BN/RijHsd and COP/OlaHsd. The albino strains were F344/NHsd, LEW/HanHsd, SHR/NHsd, WAG/RijHsd and WKY/NHsd. All strains were purchased from Harlan (Horst, The Netherlands). The health status report that was delivered with the rats indicated that they were free of the micro-organisms monitored based on Federation of European Laboratory Animal Science Associations (FELASA) recommendations. Testing of the animals commenced at the age of 12 weeks after an acclimatization period of two weeks. The protocols of the experiments were approved by the Animal Experiments Committee of the Academic Biomedical Centre Utrecht and peer-reviewed by the Scientific and Ethical Committee of the Division of Laboratory Animal Science.

Husbandry during experiment

The animals were housed in groups in Makrolon IV-S cages (2 cages/strain) with a sawdust bedding (Lignocel[®], Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). The room temperature was maintained at 20–23°C with a relative humidity of 50 ± 5% and 15–20 air changes per hour. The artificial light-dark cycle of the room was 12:12 h with lights on at 07:00 h. The rats were fed a pelleted maintenance diet (CRM (P)[®], SDS, Witham, UK) and had access to acidified (HCl) water through an automated drinking water system (pH ~ 2.5) *ad libitum*. All animals were subjected to a two-week pre-study period of habituation to being restrained in a small towel. On the day of the experiment, the strains to be tested were

placed in a room adjacent to the experimental room. During the experiment, only the animals used were placed in the experimental room, thereby minimizing potentially interfering stress responses in the other members of the strain.

Study design

Each rat was subjected to two analgesics and three anaesthetics, respectively, according to a crossover design (the animals underwent the different treatments consecutively: A-B-C-D-E; 1 drug/animal/week). A minimum washout period of one week between treatments was included with the intention of eliminating possible carry-over effects. Four experimental days were allocated to each week, and 12 animals (2 strains) were used each day. Injections and subsequent response measurements were carried out between 11:00 and 15:30 h. For practical reasons, no randomization to avoid 'day of the week' effects was attempted. All intravenous injections were performed by one and the same operator over a period of approximately 10 s. The doses used were based upon literature data (Flecknell 1996, Flecknell & Waterman-Pearson 2000). The analgesics tested were buprenorphine (Temgesic[®] 0.05 mg/kg, Schering-Plough, Amstelveen, The Netherlands; treatment 'A') and nalbuphine (Nubain[®] 1 mg/kg, Bristol-Myers Squibb, Woerden, The Netherlands; treatment 'B'). The anaesthetics tested were propofol (GABA receptor agonist) (Rapinover[®] 10 mg/kg, Schering-Plough, Utrecht, The Netherlands; treatment 'C'), medetomidine (alpha2A adrenoceptor agonist) (Domitor[®] 50 µg/kg, Pfizer, Capelle a/d IJssel, The Netherlands; treatment 'D') and ketamine (NMDA receptor antagonist) (Narketan[®] 25 mg/kg, Vétoquinol, 's-Hertogenbosch, The Netherlands; treatment 'E'). With the exception of propofol, all drugs were diluted with sterile saline to provide a suitable volume for intravenous dosing (0.2 mL/100 g body weight) (Baumans *et al.* 2001). No infections of the injection site or loss of appetite were observed during the course of the treatment period. Rats of all strains

gained in body weight at approximately the same rate throughout the experiment.

Analgesic response

A warm water tail withdrawal procedure, as described by Cook *et al.* (2000), was used to assess the animal's response to the analgesic. The rats were restrained in a small towel and the distal 7 cm of the tail was immersed into thermostat-controlled water baths. The latency to tail withdrawal was then recorded at +40°C (baseline) and at +55°C water temperature. Tests at each temperature were separated by 2–3 min, and a 15 s cut-off was imposed in all tests to minimize potential tissue damage. After baseline latencies were recorded, the analgesic was injected into the tail vein using a 25 G needle. Thirty minutes after the analgesic injection, latency to tail withdrawal from the warm water at +40 and +55°C was determined once more. The response of the rat to the analgesic was defined as the statistically significant difference in tail withdrawal latency at 55°C water temperature before and after the administration of analgesic and expressed by the $\Delta t_{55^\circ\text{C}}$. The strains could therefore be roughly divided into different response groups. The low response group had a mean $\Delta t_{55^\circ\text{C}} \leq 1.0$ s. The medium response group was defined as having a $1.0 < \text{mean } \Delta t_{55^\circ\text{C}} \leq 3.0$ s. The high response group was defined as having a mean $\Delta t_{55^\circ\text{C}} > 3.0$ s.

Anaesthetic response

The animal's response to the anaesthetic was assessed by measuring 'sleep time' (Gruber *et al.* 1954). The rats were restrained in a small towel and injected with the anaesthetic in the tail vein using a 25 G needle. The time between injection and loss of righting reflex (latency) was noted. After the animals had lost their righting reflex, they were placed in dorsal recumbence on a heating pad that was maintained at body temperature. Ophthalmic ointment (Duodrops, Céva Santé Animale, Naaldwijk, The Netherlands) was applied to the eyes to prevent corneal desiccation or abrasions. As

soon as the animal was able to perform three consecutive righting reflexes, this was considered the time of regain of righting reflex and noted accordingly. The time frame between loss and regain of righting reflex was thereby considered the sleep time of the animal.

Statistical analyses

Results are presented as means \pm SD. All data were tested for normality using the Kolmogorov-Smirnov one-sample test. All results within groups were found to be normally distributed. Student's *t*-test for paired data was used to evaluate changes with time within strains. For comparison of group data, the Kruskal-Wallis test was used. If the Kruskal-Wallis test showed significant effects, the group means were further compared with Scheffé's test. Strain comparisons of the time from injection to loss of righting reflex for propofol and ketamine were not performed as all animals lost their righting reflex upon injection. A subsequent comparison of means for the sleep times between the pigmented and albino strains was performed using the unpaired Student's *t*-test. The probability of a type I error <0.05 was taken as the criterion of significance. Two-side probabilities were estimated throughout. All statistical analyses were carried out according to Petrie and Watson (1995) using the SPSS computer program.

Results

Analgesic response

At 40°C water temperature, all strains failed to withdraw their tail by the 15 s cut-off time before and after the administration of buprenorphine and nalbuphine (results not shown).

At 55°C water temperature, no significant differences were found in tail withdrawal time between the strains when no analgesic was administered. After administering buprenorphine, the ACI, BN, COP, LEW, SHR and WAG strains showed a significant increase in tail withdrawal time. None of the strains exhibited significant increases in tail withdrawal time after administering nalbuphine (Table 1). Figure 1 shows the $\Delta t_{55^\circ\text{C}}$ of buprenorphine for the eight strains. The effect of buprenorphine was strongest in the ACI strain ($\Delta t_{55^\circ\text{C}} = 12.0 \pm 1.3$ s), while the effect in F344 and WKY strains did not reach statistical significance.

Anaesthetic response

Propofol induced loss of righting reflex in all strains immediately after administration. Anaesthesia and subsequent recovery were uneventful. After recovery, all animals quickly regained their ability to move around the cage. Strain F344 had the longest sleep time (16.17 ± 1.94 min), while COP had the shortest (7.33 ± 1.21 min; Table 2).

Table 1 Time interval between immersion and tail withdrawal in 55°C water temperature before and after the administration of analgesic[†]

	Before buprenorphine (s)	After buprenorphine (s)	Before nalbuphine (s)	After nalbuphine (s)
ACI	2.0 \pm 0.6	14.0 \pm 1.7**	2.5 \pm 0.6	2.7 \pm 0.5
BN	2.3 \pm 0.5	3.3 \pm 0.5*	2.3 \pm 0.5	2.0 \pm 0.0
COP	2.0 \pm 0.0	3.3 \pm 0.5*	2.0 \pm 0.0	2.5 \pm 0.6
F344	2.3 \pm 0.5	3.8 \pm 1.3	1.8 \pm 0.4	2.2 \pm 0.4
LEW	2.0 \pm 0.6	2.7 \pm 0.5*	2.0 \pm 0.0	1.7 \pm 0.5
SHR	2.3 \pm 0.8	4.7 \pm 1.6*	2.8 \pm 0.8	2.3 \pm 0.5
WAG	1.8 \pm 0.4	3.2 \pm 0.8*	1.0 \pm 0.0	1.0 \pm 0.0
WKY	2.0 \pm 0.6	2.5 \pm 0.8	1.8 \pm 0.4	1.8 \pm 0.4
Significance [‡]	—	S	—	—

Asterisks indicate significant differences in values before and after the administration of analgesic (* $P < 0.05$, ** $P < 0.005$; paired Student's *t*-test)

[†]Values are means \pm SD for six rats per strain

[‡]Significance based on Kruskal-Wallis test ($P < 0.05$). S=significant strain effect

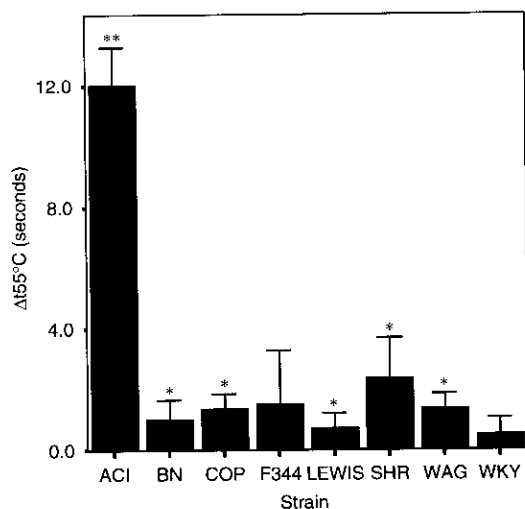


Figure 1 $\Delta t_{55}^{\circ}\text{C}$ using buprenorphine in eight rat inbred strains. Each diagram represents mean value \pm SD (6 rats/strain). The numbers in the bars represent the mean value. There are significant strain effects ($P \leq 0.0005$, Kruskal-Wallis test). There are significant differences between values before and after the administration of buprenorphine (* $P < 0.05$, ** $P < 0.0005$; paired Student's t -test). The ACI inbred strain differs significantly from the other strains ($P < 0.05$; Scheffé's test)

After the administration of medetomidine, all strains except BN lost their righting reflex. The latency (time between injection and loss of righting reflex) was most apparent in the F344 strain (11.17 ± 6.82 min; Table 2). The other strains lost their righting reflex significantly faster (Table 2). The animals of the BN strain did not lose their righting reflex at all, even after repeated efforts to place the animals in dorsal recumbence. The latter was attempted well beyond the maximum time interval between injection and loss of righting reflex as observed in the other strains. All seven strains that lost their righting reflex recovered without incident from medetomidine anaesthesia. Upon placement back in their home cage, the animals often retained an unstable posture or fell back into a state of sedation for some time.

Sleep time varied significantly among the strains, with the longest time for the LEW strain (75.83 ± 16.13 min). The F344 strain, while having the longest latency, regained

Table 2 Latency (time between injection and loss of righting reflex) for medetomidine and sleep times for propofol, medetomidine and ketamine in eight rat inbred strains

Drug	Inbred strains								P value*
	ACI	BN	COP	F344	LEW	SHR	WAG	WKY	
Propofol									
Sleep time	8.50 ± 3.27^a	9.17 ± 1.47^b	7.33 ± 1.21^c	16.17 ± 1.94^{abcde}	10.83 ± 2.48^d	9.50 ± 1.05^e	8.17 ± 3.87^f	12.00 ± 1.10	< 0.0005
Medetomidine									
Latency	1.67 ± 0.52^a	nr	3.17 ± 1.33^b	11.17 ± 6.82^{abcde}	1.67 ± 0.52^c	2.67 ± 1.75^d	2.00 ± 0.63^e	3.00 ± 0.63^f	< 0.0005
Sleep time	55.50 ± 7.79^a	nr	60.50 ± 12.53^b	15.67 ± 9.16^{abcde}	75.83 ± 16.13^c	59.67 ± 9.22^d	71.83 ± 7.68^e	48.83 ± 6.49^{ce}	< 0.0005
Ketamine									
Sleep time	na	na	20.00 ± 1.27^a	16.83 ± 3.60^{bcd}	19.83 ± 4.02^e	26.33 ± 1.63^{abe}	23.50 ± 3.45^c	25.83 ± 2.48^d	< 0.0005

Each value represents mean \pm SD (6 rats/strain) in minutes. na=not available, nr=no response

*P value in the Kruskal-Wallis test

^aContrast significance (mean \pm SD: Scheffé's test). Within a row, values bearing the same superscript letter are significantly different ($P < 0.05$)

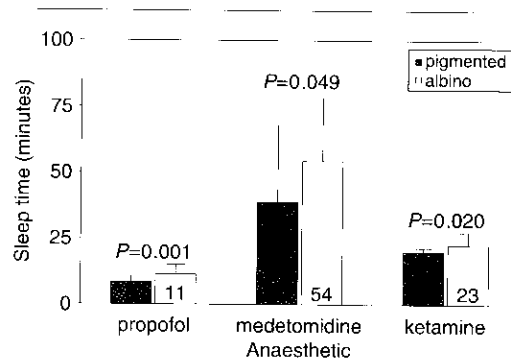


Figure 2 Sleep time comparing pigmented and albino strains using propofol ($n_{\text{pig}} = 18$, $n_{\text{alb}} = 30$), medetomidine ($n_{\text{pig}} = 18$, $n_{\text{alb}} = 30$) and ketamine ($n_{\text{pig}} = 6$, $n_{\text{alb}} = 30$). Each diagram represents mean value \pm SD. The numbers in and above the bars represent the mean and P value, respectively. Pigmented strains when compared with albino strains have a significantly shorter sleep time ($P < 0.05$; unpaired Student's *t*-test)

its righting reflex sooner than other strains (15.67 ± 9.16 min) (Table 2).

Immediately after the administration of ketamine, all strains lost their righting reflex. In the ACI and BN strains, soon after induction the mucous membranes became cyanotic and, despite resuscitation efforts, two rats of both strains died, after which the experiments using ketamine on these strains were stopped. All animals of the other strains recovered uneventfully from ketamine anaesthesia. The time needed to regain the ability to move around freely when placed back in the home cage was, however, prolonged when compared with propofol. The rats crawled unsteadily and some animals fell back into a sedative state for an indefinite period while preserving their righting reflex. Inter-strain comparison of sleep times showed significant differences, with SHR (26.33 ± 1.63 min) and F344 (16.83 ± 3.60 min) being the most contrasting (Table 2).

When comparing sleep times of albino strains with those of pigmented strains, it was found that for each of the three anaesthetics the sleep time in albino strains was significantly longer than in pigmented strains (Figure 2).

Discussion

The present study provides evidence of strain-related differences in the analgesic effectiveness to the partial μ opioid receptor agonist buprenorphine. Marked strain differences in baseline (i.e. non-drug) tail withdrawal latencies were not observed. In previous studies, significant differences were observed in baseline tail withdrawal latency between the F344 and the LEW strains even at high stimulus intensities as used in the present study (Cook *et al.* 2000). In accordance with the present study, other studies could not confirm these results using the same strain and stimulus intensity (Barrett *et al.* 2002). Six out of eight strains showed significant increases in tail withdrawal latencies subsequent to the administration of buprenorphine, with ACI as the most contrasting responder. This analgesic effect of buprenorphine in ACI is not in line with an earlier study by Turner *et al.* (2003), who found that ACI was only moderately sensitive to buprenorphine. Since the strain in the present study was supplied by the same vendor, we have as yet no explanation for the observed discrepancy. The BN and LEW strains showed low responsiveness to the analgesic effects of buprenorphine, which confirms previous studies (Turner *et al.* 2003). Since the ACI and BN strains were subjected to buprenorphine administration on the same day, confounding 'day of the week' effects seem unlikely as rats of the BN strain responded quite differently to rats of the ACI strain. The F344 and the WKY strains were found to be unresponsive to the analgesic effects of buprenorphine at the dose rate used. Previously, the F344 strain has been found to display a significant increase in analgesia following administration of buprenorphine (Cook *et al.* 2000, Turner *et al.* 2003). This discrepancy could be due to substrain differences as the rats came from a different vendor. Besides substrain differences, social stress factors during early postnatal life (Alleva *et al.* 1986, Sudakov *et al.* 1996) might have caused the difference in analgesic response. Also, differences in restraint methods should be

considered as the rats from the previous studies were placed in restraint tubes. Although a habituation protocol was followed prior to testing, it cannot be ruled out that restraint stress potentiated the analgesic effect of buprenorphine (Woolfolk & Holtzman 1995). The COP, SHR and WAG strains displayed medium responsiveness to the analgesic effect of buprenorphine at the given stimulus intensity.

In contrast with buprenorphine, nalbuphine, also representing the group of partial agonists, induced no analgesia in any of the strains under the defined experimental conditions. Others have reported the inability to define an ED₅₀ for nalbuphine at high (56°C) (Turner *et al.* 2003) or even mild (50°C) to moderate (52°C) water temperatures (Morgan *et al.* 1999, Cook *et al.* 2000). Since nalbuphine was administered at therapeutic doses in this study, one might doubt the effectiveness of nalbuphine in achieving adequate analgesia in rats at this dose level during experimental procedures.

The F344 strain responds most profoundly to the effects of propofol while the COP strain has the weakest response. The F344 strain differs markedly from all the other strains in having a substantially extended latency to loss of righting following the administration of medetomidine. It has been shown that the F344 strain possesses high levels of diurnal and stress-related corticosterone levels (Dhabhar *et al.* 1993), which might affect the distribution and metabolism of medetomidine (Raekallio *et al.* 2002) and could thus explain the delayed loss of righting reflex after administration of medetomidine. This assumption is further supported by the observation that the F344 strain regains its righting reflex substantially faster than all the other strains. The BN strain does not lose its righting reflex at all, although other signs of sedation do occur. A similar observation has previously been made in the AX/JU inbred strain of the rabbit (Avsaroglu *et al.* 2003). Further studies are necessary to ascertain whether these unrelated observations share a common mode of action.

The variation in sleep time among the strains using ketamine is less prominent

than with propofol and medetomidine. However, in the case of the ACI and BN strains, a lethal hypoxaemia occurred, which can be construed as a hyper response to the anaesthetic effects of ketamine. Administered as a mono-anaesthetic, ketamine is generally considered to be one of the few anaesthetics not to specifically suppress the respiratory rate centrally. However, it can significantly increase pulmonary viscoelastic pressure resulting in dilation of alveolar ducts and alveolar collapse (Alves-Neto *et al.* 2001). We assume that the ACI and BN strains have developed this complication more extensively than the other strains tested, but no (histo) pathological data are available to substantiate this assumption. Another explanation for the extreme response to ketamine could be found in chronopharmacological differences. Previous studies indicate that duration and magnitude of CNS depression of ketamine in rats exhibit circadian rhythmic variations (Rebuelto *et al.* 2002). Strains such as the ACI, displaying a strong 24 h circadian rhythm (Siebert & Wollnik 1991), could respond disproportionately to the depressant effects of ketamine, as Rebuelto *et al.* (2002) have shown that the longest pharmacological response occurs during the period (11:00–15:30 h) in which ketamine was administered in this study. With all three anaesthetics, the response of albino when compared with pigmented rats is higher. Whether this implies that the response to propofol, medetomidine or ketamine is governed by a genetic component that is closely linked to the albino locus on the rat chromosome can be the subject of further study.

In conclusion, the responses to buprenorphine and the anaesthetics in this study show considerable differences among the tested inbred rat strains. Results of particular interest are (a) the unusually strong response of the ACI rats to buprenorphine; (b) the relative resistance of the F344 and BN rats to medetomidine; (c) the relative hypersensitivity of the ACI and BN rats to ketamine. It would be of interest to study the genetic basis of the differences in response. A prerequisite for finding QTLs with a

significantly high LOD score is that the assessment of the phenotype is accurate and reproducible. This study has addressed a number of confounding influences on the response to anaesthetic and analgesic drugs. However, although refining of the methodology might further increase the accuracy of phenotyping, the results so far seem to be sufficiently discriminative as to provide a solid basis for the design of a QTL study.

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