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Long-term consequences of repeated pentobarbital anaesthesia on choice reaction time performance in ageing rats

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Recent studies have suggested that anaesthesia may be a factor in cognitive decline with age. We examined the effect of repeated (eight times) anaesthesia with pentobarbital on reaction time performance in rats in a longitudinal study. Treated rats had faster response times and made more premature responses than the control rats when they were older than 21 months. The results suggest that repeated anaesthesia during the lifespan can lead to an increase in impulsivity, as assessed by a choice reaction time test, during the later stages of life in the rat. These findings support the theory that repeated anaesthesia is a biological factor that affects cognitive ageing.

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Several studies have shown that patients who undergo anaesthesia and surgery may develop a cognitive deficit in the postoperative period and that age is a major risk factor. These impairments seem to be short-lasting and generally disappear after several days to weeks,[1–3] although two large-scale studies have shown that 5–10% of elderly patients show a cognitive deficit that persists for several months[4 5] and may be permanent.[6]

The many, uncontrollable variables in human subjects make appropriate studies difficult and an animal model would be helpful. We describe a longitudinal experiment in which the effects of repeated anaesthesia on reaction time were examined in rats. We repeatedly anaesthetized and tested their cognitive performance with a choice reaction time (CRT) task throughout life. The study continued until the rats were 26 months old, when rats may be considered 'aged'. If anaesthesia is a vulnerability factor for cognitive ageing, the performance of the experimental groups should diverge in the course of their lifespan.

Methods and results

Animals

All experimental procedures were approved by the research ethics committee of Maastricht University for animal experiments and met government guidelines. Twenty-seven Lewis rats were obtained from the animal facilities of Maastricht University. The animals were housed individually in standard Makrolon cages on sawdust bedding in an air-conditioned room (about 20°C) under a 12/12-h reversed light/dark cycle (lights on from 18.00 to 6.00 h) and had free access to food and water.

Experimental design

At the age of 6–8 months, the rats were trained on the CRT task[7] after food deprivation to 85% of free feeding weight, matched for their reaction time performance and assigned to the control (n=12) or treatment (n=15) group. Rats in the treatment group were then anaesthetized and the CRT was repeated 2 days later. Free feeding was then permitted until the next test session. The control group was treated and tested identically apart from the anaesthesia. Thereafter, the sequence of food deprivation, training, CRT, anaesthesia and CRT was repeated at intervals of 2.5 months, when the rats were 6, 8.5, 11, 13.5, 16, 18.5, 21 and 23.5 months old. Two months after the last study period the CRT was repeated for the last time, as the number of rats that responded reliably in the behavioural task was reduced to eight and nine in the control and treatment groups respectively. After the final behavioural test, when the rats were 26 months old, the animals were killed and the brain was removed for biochemical analysis.

Anaesthesia

Sodium pentobarbital (20 mg kg^{-1} in 0.3 ml) was administered by i.p. injection. Qualitative observations indicated that the animals lost their reflexes and were unresponsive to gentle stimuli for about 2–3 h. No surgery or painful stimuli were administered. During the period of anaesthesia the rats were placed in an incubator held at a constant 32°C. When the rats had recovered, they were returned to their home cage. Control rats were not injected.

Statistical analysis

All data were compared by analysis of variance with two factors (treatment and age), with age as a repeated-measures factor. Group differences at individual time points were evaluated using the *t*-test.

Mortality

Four control rats and four rats from the anaesthetic group died for unknown reasons. Of the remaining rats, two rats in the anaesthetic group did not complete sufficient trials in the behavioural tasks and were excluded from the statistical analyses.

Reaction time performance

Reaction time

Figure 1A shows the change in reaction time with age. The rats responded faster with age up to the age of 18.5 months [age, $F(5,75)=6.89$, $P<0.01$], and this was similar for the two experimental groups [treatment × age, $F(5,75)=1.55$, not significant]. Individual *t*-tests revealed a tendency for a faster mean reaction time in the treated group in the last two test sessions ($t<1.96$, $0.05<P<0.10$).

Motor time

In contrast to reaction time, motor time did not change over the first six test sessions [age, $F(5,75)=1.07$, not significant] (Fig. 1B) and there were no differences between groups (treatment × age, treatment $F<0.89$, not significant). Motor time increased during the last three test sessions [age, $F(2,30)=213.13$, $P<0.01$], but the groups did not differ [treatment × age, $F(2,30)=2.19$, not significant].

Premature responses

The development of premature responses is shown in Fig. 1C. There were no statistically reliable differences between the groups for the first six test sessions (treatment × age, treatment $F<1.32$, not significant). However, for the last three test sessions there was a tendency for treated rats to make more premature responses than control rats [treatment, $F(1,15)=3.64$, $0.05<P<0.10$], which was due mainly to the treatment effect in the last test session [$t(15)=2.79$, $P<0.05$].

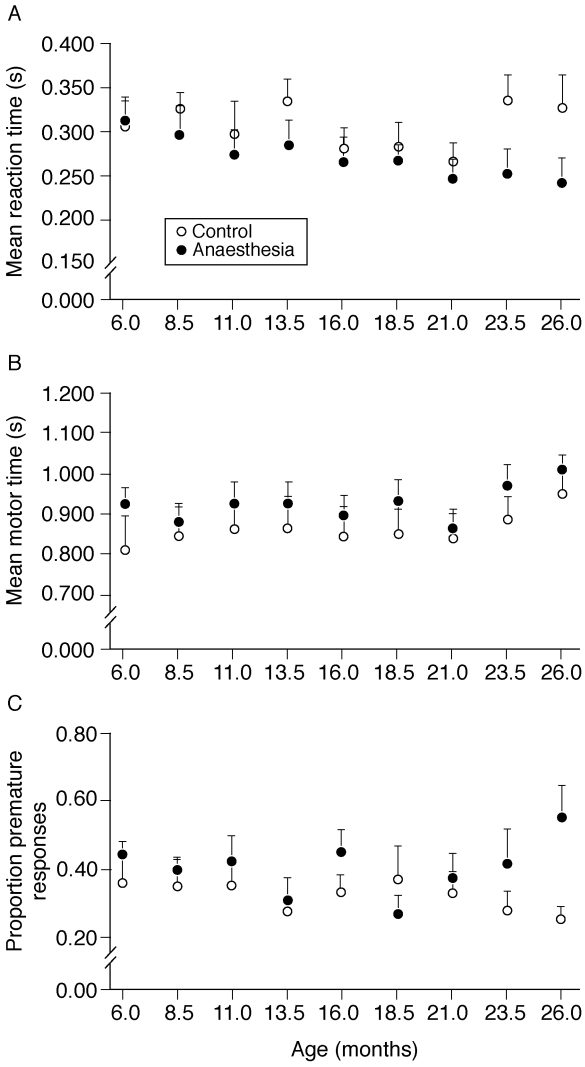

Fig 1 Performance of control and anaesthetized rats in a reaction time task tested at several time-points during their lifespan. (A) Mean reaction time. (B) Mean motor time. (C) Proportion of premature responses. Values are mean and SEM of two sessions.

Comment

We showed that repeated anaesthesia throughout life began to affect the performance of a reaction time task when the rats were over 21 months old, i.e. aged. The treated group tended to react faster than the control group during the last two test sessions. Furthermore, the anaesthesia group made more premature responses during the last test session. Faster reaction times together with an increase in errors (i.e. premature responses) is usually interpreted as increased impulsivity.[8]

Previous human studies that have shown neurological changes have noted that the observed effects may have resulted from either the anaesthetic or the accompanying surgery and the associated stress response or both.[1] No surgery was conducted on the animals in the present study

and, while we cannot exclude a degree of stress, the likely cause of the observed changes was the pentobarbital.

It remains to be determined whether the effects of treatment in the present study were related to an acute effect of pentobarbital in old rats or were the result of a cumulative effect. Acute thiopental treatment did not affect the spatial memory performance in young and old rats (given the same dose of thiopental), which does not support the concept that the effects in the present study were an acute effect of treatment.[9] Moreover, in the present study the rats were not anaesthetized before the final behavioural test, when behaviour was most affected, strongly suggesting that the effects of anaesthesia are long-lasting or cumulative.

In summary, our findings suggest that repeated anaesthesia affects the behaviour of rats in the later stages of life through an increase in impulsivity. The findings support observations in studies with healthy old people[10] and aged patients[1–5] and supports the hypothesis that repeated anaesthesia is a vulnerability factor for cognitive ageing. Further studies examining the effects of different anaesthetic agents and different patterns of administration in additional animal models are indicated.

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