

Chronic aspirin ingestion improves spatial learning in adult and aged rats

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Abstract

Epidemiological evidence suggests that nonsteroidal, anti-inflammatory drugs (NSAIDs) may retard the progression of Alzheimer's disease (AD). In the present study, we have chronically treated adult (4–5 months old) and aged (20+ months) rats with water adulterated with aspirin, and examined spatial learning in a swim maze. Adult rats ($n=40$) and aged rats ($n=20$) were divided into separate groups assigned to receive either normal drinking water or water with 2 mg/ml of aspirin dissolved in it. For 6 weeks, we monitored daily water and/or drug intake before testing all rats in a standard swim maze over an 8-day period. On average, each rat drank approximately 25 ml of water/day with no apparent control versus aspirin group differences. There was no effect of aspirin in young adult rats except during a visible platform trial where aspirin-treated rats performed better than controls. In contrast, aspirin markedly improved performance in the aged rats during hidden and visible platform trials. Such group differences abated by the eighth test day when all rats performed equally well. The improvements in performance were not correlated with changes in swim speeds indicating that the enhancement was not due to facilitated motor output. These data reveal that a modest, 6-week treatment regimen with aspirin in aged rats is sufficient to induce improvements in both speed of learning and strength of the learned response. We have yet to address the key question as to underlying physiological mechanism(s) that might underpin this augmented cognitive performance. Moreover, it would be useful to ascertain whether or not chronic NSAID treatment might reduce the extent of learning impairments in aged, cognitively impaired animals. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

Epidemiological evidence has identified a possible prophylactic action, or at least an ameliorative effect, of nonsteroidal anti-inflammatory drug (NSAID) use on the incidence and expression of Alzheimer's disease (AD) (Breitner, 1996). Not all studies have positively correlated NSAID use with AD onset (Jenkinson et al., 1989; McGeer et al., 1989), although many of the discrepancies are probably due to definitions of NSAID use (concurrent and past), duration of use, dose, and whether or not patients could reliably recall details about NSAID use or if accurate monitoring had occurred in a clinical setting. Taking such factors into account, the relative risk for developing AD is markedly lower in those subjects aged under 85 years who also used NSAIDs for 6 months or

more in the preceding year (In 't Veld et al., 1998; Launer and In 't Veld, 1998). One of the underlying assumptions that arises from such observations is that neuronal inflammation may appear as a contributing factor in either the onset or severity of AD pathology. Indeed, a substantial body of evidence shows that proinflammatory cytokines are overexpressed in brain tissue samples from AD patients (Dickson et al., 1993; Mrak et al., 1995) and that regular use of NSAIDs reduces the amount of astrocytes and tends to diminish the number of activated microglia in AD patients (Alafuzoff et al., 2000).

The prototypical NSAID is aspirin or acetylsalicylic acid, which is the *o*-acetyl derivative of salicylate, the active ingredient (Vaino and Morgan, 1997). Salicylates are found in relatively large quantities in willow bark, and concoctions made from brewing the bark were used thousands of years ago. NSAIDs inhibit the formation of prostaglandins, and this forms the basis for their analgesic and anti-inflammatory actions (Appleton et al., 1996; Vane and Botting, 1998). Prostaglandins are metabolic byproducts of the breakdown of arachidonic acid, a process that

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relies on cyclooxygenase (COX). There are two forms of this enzyme, a constitutive isoform, COX1, and an inducible isoform, COX2. COX1 is thought to regulate prostaglandin production in areas such as the stomach where it acts to protect the lining (thereby explaining the adverse GI effects of aspirin) and COX2 is induced by inflammatory cytokines and is found in brain and spinal cord (Appleton et al., 1996; Vane et al., 1998).

As might be expected, the hypothesis that inflammation is a central feature of AD is supported further by studies looking at COX activities in diseased brains. In temporal cortex samples from AD patients, COX1 activity is elevated in cytosolic and particulate fractions, while COX2 protein is elevated in particulate fractions only compared to nondemented control brains (Kitamura et al., 1999). Immunohistochemical studies using temporal and frontal cortex tissues found that microglia, positive for COX1 expression, were related to the presence of β -amyloid plaques, while COX2 expression was principally neuronal in origin, but elevated in AD brains compared to controls (Hoozemans et al., 2001). Thus, the biochemical basis explaining why NSAID use protects against AD may relate to their ability to reduce COX1 and COX2 activities that are elevated by inflammatory cytokines induced by the presence of aberrant proteins such as β -amyloid. Furthermore, the DNA binding transcription factor, PPAR-G, may also be stimulated by NSAIDs and inhibit the expression of COX2 and reduce the β -amyloid-induced secretion of microglia-proinflammatory products (Combs et al., 2000).

Surprisingly, there appears to be very little information about aspirin use and cognitive function in animal studies; indeed, behavioural experiments looking at aspirin appear to focus only on pain mechanisms (e.g., LaBuda and Fuchs, 2001), although its ability to target the arachidonic acid cascade has prompted further use (Yamaguchi et al., 2001). We thought it to be worthwhile to investigate whether or not aspirin might modify spatial behaviour of rats in a swim maze, and cognizant of its actions in elderly, human demented subjects, whether any age-related effects might predominate. Moreover, performance in the water maze is particularly dependent on intact hippocampal function, a structure that shows considerable degeneration in AD, and therefore the swim maze is a more useful tool to assess empirically whether or not NSAIDs might be usefully administered to AD patients (Fibiger, 1991; Morris et al., 1982; O'Keefe and Nadel, 1978). We have also chosen the water maze to assess cognitive function since this task is particularly sensitive to disruptions with age.

2. Methods

2.1. Subjects

Sixty adult male, Lister hooded rats (450–600 g) bred at the University of Bradford, were used as experimental

subjects. Forty subjects were 4–5 months of age, while the remaining 20 were from our ageing colony. The aged rats (19–24 months old) had served as breeders until being retired at 1 year of age and left in group conditions until use. All rats were housed in groups of three to five in clear, polycarbonate cages (40 × 60 × 17 cm) with stainless steel lids. Standard rat chow (Purina) and tap water were provided *ad libitum*. The colony room was environmentally controlled with an ambient temperature of 20–22 °C and a relative humidity of approximately 65%. The rooms were kept on a normal light cycle with lights on at 08:00 h and off at 20:00 h. Cage maintenance was undertaken twice weekly, but never prior to behavioural testing.

2.2. Apparatus

The water maze consisted of a circular pool approximately 150 cm in diameter and painted white. The water depth was kept at 30 cm and the water was coloured opaque using a latex solution to mask the location of the escape platform; the water temperature was maintained at 30 °C. The escape platform was 10 cm in diameter and submerged 3 cm below the surface of the water. A heavy, black curtain surrounded the entire maze to reduce distractions to the animal from experimenter movements. The walls of the maze enclosure had visual cues positioned around the pool, consisting of poster boards with broad black and white lines drawn either horizontally or vertically. Suspended above the pool about 180 cm from the water surface was a video camera that was attached to a video tracking system and computer analysis program. The computer was programmed to record path lengths travelled and latencies to locate the platform for each trial.

2.3. Behavioural testing

The testing regime was carried out over an 8-day span. On each test day, rats were placed onto the submerged platform for 10 s in order to form associations between the position of the platform and the various visual cues around the pool. The platform was always kept in Quadrant 3. The rat was then lowered into the pool, facing towards the wall in Quadrants 1, 2, or 4 (chosen at random) and allowed to swim for a maximum of 60 s, or until it had located the platform. If the rat was successful in locating the platform, it was permitted 10 s on the platform before being positioned in the water in another quadrant (again chosen at random). Each rat was subjected to a total of four trials. Rats unable to locate the platform on any trial were always positioned on the platform for 10 s prior to any further trials. On the final trial (trial 5), the submerged platform was replaced with a visible platform that projected 3 cm above the surface of the water to test their ability to learn an escape task. At the conclusion of testing, rats were dried under a heat lamp and returned to their home cages. This procedure was repeated on Days 2, 3, 4, and 8 using identical protocols.

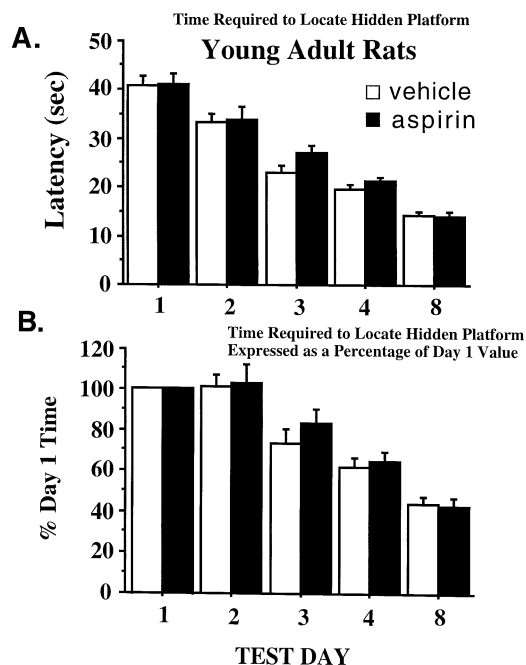


Fig. 1. The effects of chronic aspirin treatment in young adult rats on times to locate a hidden platform in a swim maze task. (A) Actual data obtained. (B) Data from (A) are expressed as a percentage of Day 1 performance times. Overall, there were no significant effects of aspirin on performance of this task; both vehicle- and aspirin-treated rats showed progressively better performance over the 8-day testing period. Means \pm S.E.M. are shown in (A).

2.4. Drug treatments

Six weeks prior to testing, rats were randomly assigned to groups that received either normal drinking water or drinking water with 2 mg/ml aspirin dissolved in it. Rats normally drink 20–40 ml of water/day (depending on room temperature) and this ensured that each animal received approximately 40–80 mg/day aspirin (an amount similar to that used in humans—75 mg—treated as a prophylactic against heart attacks). We monitored water intake for all groups and weight changes to ensure that diet and hydration were similar for both groups. There were no obvious changes in either body weights or amount of liquid consumed by aspirin or water-treated animals.

2.5. Drugs

Aspirin was purchased from the Sigma (UK) and prepared fresh each day by dissolving in ordinary drinking water. The concentration of aspirin used was 2 mg/ml, which eventually dissolved with intense stirring.

2.6. Statistical analysis

Latencies (in seconds) to locate the hidden or visible platform were assessed by a split-plot factorial analysis of

variance (ANOVA) with pretreatment (vehicle or aspirin) as an orthogonal factor and test day as the repeated measure factor. In order to facilitate analysis, the test trials were averaged to give a single daily score. In order to control for violations of sphericity of the variance/covariance matrix, the degrees of freedom for the repeated-measures factor are reported as N (overall sample number) – $(K - 1)$ (number of group combinations). This is commonly referred to as a Greenhouse–Geisser correction and prevents the inflation of the degrees of freedom associated with repeated-measures designs. Post hoc tests were made using Bonferroni-corrected t tests designed to maintain the experimentwise alpha level at $P < .05$ if the main or interactive ANOVA effects were significant (Bray and Maxwell, 1982).

2.7. Ethical statement

All procedures carried out in this report conform to the requirements of the UK Animals Scientific Procedures Act 1986, under project license number ppl-50/1974.

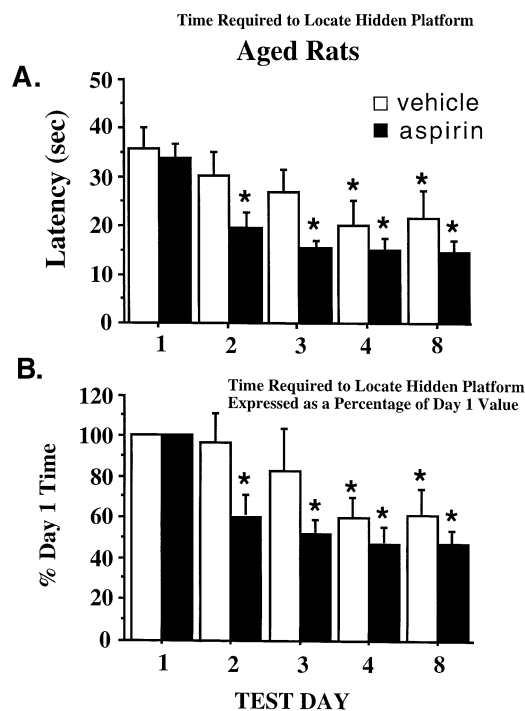


Fig. 2. The effects of chronic aspirin treatment in aged rats on times to locate a hidden platform in a swim maze task. (A) Actual data obtained. (B) Data from (A) are expressed as a percentage of Day 1 performance times. Both vehicle- and aspirin-treated rats showed progressively enhanced learning over the 8-day test period. Furthermore, the rate of learning was increased by aspirin ingestion as shown by the significant improvement in learning seen on Days 2 and 3, but not observed in vehicle-treated animals. The performance of all rats was significantly improved by Test Day 4 as compared to Day 1. Means \pm S.E.M. are shown in (A). * Significantly different from corresponding group compared to Day 1 of testing ($P < .05$).

3. Results

3.1. Latency to locate hidden platform

There were no overall effects of aspirin on latencies to locate the hidden platform in young adult rats, nor any interactions between day of testing and drug treatment. There was an expected effect of test day, $F(4,39)=86.9$, $P<.05$, indicating that all animals learnt the task over the testing period. These results are shown in Fig. 1A, while Fig. 1B shows how performance over the test days appeared when expressed as a percentage of Day 1 behaviour. Both aspirin- and vehicle-treated rats showed a progressive improvement in learning the task and, on Day 8 of testing, showed similar retention of orientation in the swim maze. However, these data contrast sharply with those of the aged rats. ANOVA performed on the data obtained from aged rats revealed significant main effects of test day, $F(4,19)=6.47$, $P<.05$, showing that both groups learn the swim maze over time; there was also a significant effect of drug treatment, $F(1,19)=7.89$, $P<.05$. Surprisingly, the interaction between day of testing and drug treatment was not significant,

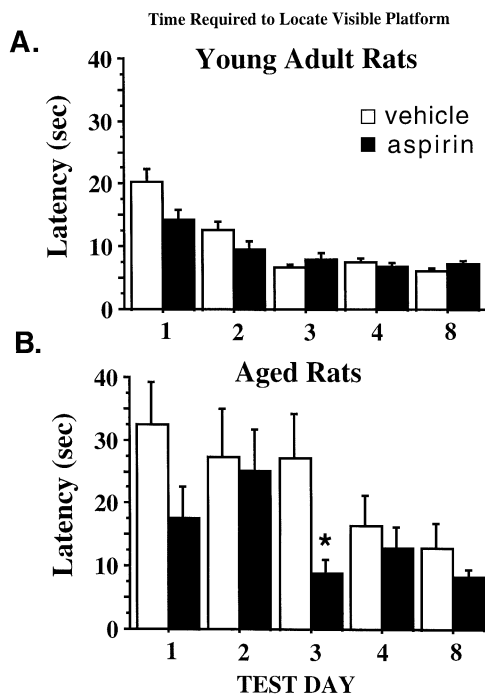


Fig. 3. The effects of chronic aspirin on times to locate a visible platform in adult rats (A) or in aged rats (B). Overall, young adult rats that received aspirin were quicker to locate the platform and make an escape response, an effect that was most evident on Days 1 and 2 of testing. However, pairwise comparisons failed to show any particular test day effect. Similarly, aged rats that received aspirin also swam to the visible platform more quickly than did vehicle-treated animals. Interestingly, the variability in the data was somewhat higher in the aged rats compared to the young adult animals, and overall aged rats were slower to locate the visible platform compared to young rats. Means \pm S.E.M. are shown. *Significantly different from vehicle-treated group on the same test day ($P<.05$).

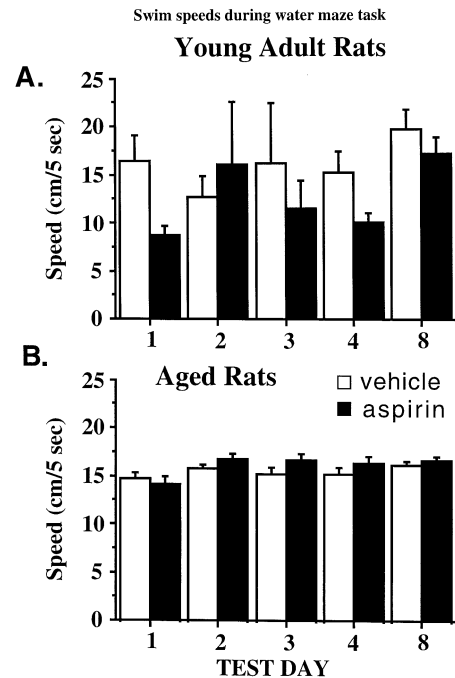


Fig. 4. The effects of chronic aspirin on swim speeds during a water maze task in adult rats (A) or in aged rats (B). Overall, there were no significant effects of aspirin on swim speeds at either age, or on any particular test day. Means \pm S.E.M. are shown.

$F(1,19)=0.47$, ns. As shown in Fig. 2A, aspirin-treated rats exhibited improved learning from Day 2 onwards compared to the vehicle-treated rats who showed significant improvements by Day 4. By Day 8 of testing, all rats were approximately similar, and although aspirin-treated rats appear slightly more proficient at locating the platform, pairwise comparisons do not show any significant effects on the respective test days. These data are also shown in Fig. 2B, where the values are expressed as a percentage of Day 1 performance. Here, we see stronger evidence for the cognitive enhancing properties of aspirin in aged rats.

3.2. Latency to locate a visible platform

Whereas aspirin had little obvious effect in young adult rats on their performance in the swim maze with a submerged platform, there were interesting effects of aspirin on performance when a visible platform was used. ANOVA on these data revealed a significant main effect of drug treatment, $F(1,39)=4.7$, $P<.05$, which can be summarised by stating that overall aspirin-treated rats were quicker to locate the visible platform and escape the water. The interaction between drug treatment and test day just failed to meet significance, $F(4,39)=3.98$, $P<.07$, although inspection of Fig. 3A shows that aspirin tended to facilitate escape behaviour principally on test Days 1 and 2. On Days 3, 4, and 8, both groups were equally proficient at locating the visible platform, almost as soon as they were placed into the water. ANOVA performed on the latencies to locate

the visible platform obtained from aged rats revealed a significant main effect of drug treatment, $F(4,19)=7.65$, $P<.05$. As shown in Fig. 3B, over all test days aspirin-treated rats exhibited faster escape latencies compared to vehicle-treated rats, an effect that was in evidence from Day 1. Moreover, by Day 8, aspirin-treated rats appeared to be better at escaping the water compared to the vehicle controls, although individual test day comparisons only showed a significant effect on Day 3.

3.3. Swim speeds

Swim speeds were calculated by sampling path lengths and measuring the time spent to move a specific distance. Fig. 4A shows the data obtained for the young adult rats. ANOVA on these data revealed no significant effects of aspirin treatment, nor any interactive effects. Generally, swim speeds were comparable for both aspirin- and vehicle-treated animals, and although there was quite a bit of variability over the various test days, there was no particular pattern that emerged. Interestingly, the aged rats also exhibited consistent swim speeds regardless of group membership, and independent of any test day effects. These data are depicted in Fig. 4B. However, the aged rats showed remarkable test day concordance in that swim speeds remained very stable for both aspirin and vehicle-treated rats over all test days.

4. Discussion

These data reveal that a very modest treatment regimen with aspirin in rats is sufficient to induce obvious improvements in the speed of learning. The improvement in cognitive ability was most evident in aged rats tested with a submerged platform, where aspirin-treated animals performed better, although young adult rats given aspirin performed better overall during the visible platform test. It is possible that the younger rats are not generally responsive to aspirin because they already perform to a high level (i.e., a ceiling effect), whereas the aged rats are somewhat less capable of learning and aspirin is able to induce enhanced performances. It is worthwhile noting that improved learning abilities are not due simply to enhanced motoric output (thereby increasing the likelihood of locating the submerged platform by chance alone), since such hyperactivity might be expected to impair performance during the visible platform test. Moreover, measures of swim speeds were comparable for vehicle- and aspirin-treated rats suggesting that the improvements seen in the aged rats in particular were not merely due to improved motor output or limb flexibility.

The age-specificity of aspirin that we report in this paper is mirrored by preliminary trials in human subjects. The use of NSAIDs in nondemented, middle-aged subjects has failed to provide much substantiation for any cognitive-enhancing properties of these drugs (Peacock et al., 1999), although

geriatric subjects (mean age 77.4 years) do show enhanced mental abilities (Bertozzi et al., 1996). Moreover, there also appears to be a protective effect of chronic NSAID use against cognitive decline in elderly subjects (Prince et al., 1999; Rozzini et al., 1996; Sturmer et al., 1996). The caveats discussed in the introduction are worth reiterating, however, since these studies rely on subjective accounts of NSAID use, and fail to detail how much of the drug is actually being taken. Most of the research groups comment on the need for randomised, double-blind trials to measure the veracity of the claims for procognitive actions of NSAIDs.

Exactly how aspirin and other NSAIDs might influence cognitive function is unclear. Obviously, if there is a known inflammatory condition that may be affecting neuronal function then NSAIDs could act to reduce this. In the case for AD, this may well represent the mode of action as detailed in the introduction. It is interesting to note that steroidal anti-inflammatory drugs have markedly different effects on neuronal function; indeed these agents put neuronal tissue at risk and may exacerbate age-related cognitive decline (Meaney et al., 1993; Sapolsky, 1992). This might be the result of their catabolic actions, which in the long term are detrimental, actions that NSAIDs do not possess. Furthermore, acute blockade of hippocampal corticosteroid receptors actually enhances cognitive function (Oitzl et al., 1998; Smythe et al., 1997).

Human dementia is caused by a multifaceted array of problems and, in particular, can result from AD, and vasculature conditions such as infarcts and ischaemia (Stoppe et al., 1996). Of the NSAIDs currently in widespread use, aspirin has potent effects on the vasculature and blocks platelet aggregation; indeed, aspirin is often the first drug of choice for antithrombotic therapy in vascular dementia (Molnar et al., 1998). It is not inconceivable that aspirin enhances function or retards cognitive decline through an action on brain vasculature (enhancing blood flow, reducing ischaemic attacks, and so on). Younger animals and younger human subjects would be less prone to vascular disruptions and this would explain the relative lack of effect of aspirin in younger human subjects. Of course, this does not preclude its actions to enhance cognition by reducing neuronal inflammation if it is present, as hypothesised in AD (Breitner, 1996).

The present data are preliminary and based on a restricted design with relatively few animals included in the study. Moreover, we have yet to address the key question as to underlying physiological mechanism(s) that might underpin this augmented cognitive performance. Further issues to be addressed regarding cognition and NSAIDs include: time course and dosages and whether or not vasculature or inflammatory mechanisms are involved. Highly selective COX2 inhibitors might also be worth testing since these have no GI effects. Finally, it would be worthwhile to investigate whether or not NSAIDs might act to reduce deficits in aged, cognitively impaired animals, a better model of age-related, human dementia.

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