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Facilitation of a weak training experience in the 1-day-old chick using diphenylhydantoin: a pharmacological and biochemical study

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Abstract

This series of studies used a weakly trained (20% methyl anthranilate) version of the passive avoidance learning task in the 1-day-old chick to investigate memory facilitation effects by diphenylhydantoin (DPH). The results indicated that the pairing of the weak training experience with DPH results in facilitation of memory that can be observed from 40 min following training with the weak training experience. The results from a biochemical experiment indicated that DPH facilitates the activity of Na^+/K^+-ATPase at the majority of times sampled in a large percentage of the sections of the chick brain. The most marked level of elevation in the activity of the enzyme was observed at the 20-min time point following weak training in the section of the chick brain, which contained several memory relevant neuroanatomical loci. This represents a 68% increase in the activity of the enzyme in those areas considered to be crucial to the processing of memory in the paradigm at a time predicted by previous investigation to be crucial in the development of the intermediate-term memory stage of memory. The results of this series of studies support the notion that Na^+/K^+-ATPase plays an important role in memory processing following passive avoidance training in the 1-day-old chick.

Keywords: Diphenylhydantoin; Weak training experience; Na^+/K^+-ATPase

1. Introduction

The Gibbs and Ng, 1977; Ng and Gibbs, 1991) model of memory proposes three sequentially dependent stages of the memory formation process following passive avoidance training with the 1-day-old chick. The second of these stages, intermediate-term memory (ITM), lasts from ~ 20 to 50 min following training, and its formation is attributed to neuronal hyperpolarization induced by Na^+/K^+-ATPase (or the sodium pump). Na^+/K^+-ATPase blockers such as the cardiac glycoside ouabain and ethacrynic acid inhibit the formation of ITM and induce retention deficits that become apparent 15 min following training.

In 1-day-old chicks trained in our laboratory on the passive avoidance task, strong training [i.e., training with concentrated methyl anthranilate (MeA) used as aversant] yields high retention levels for at least several days after the learning experience. In contrast, weakening the level of aversiveness of the stimulus [i.e., training with a 20% (v/v) dilution of MeA in ethanol] results in high retention levels only for the first 35–40 min following learning, with retention returning to baseline levels thereafter (Crowe et al., 1989a).

Results from our laboratory (Crowe et al., 1989a,b, 1990, 1991a,b) as well as those from other investigators (Johnston and Rose, 1998) have indicated that the weak learning (WL) experience can be strengthened to the equivalent of a strong learning experience by induction of the acute stress response closely in time to the weak training experience by the application of agents such as noradrenaline (NA), adrenocorticotropic hormone (ACTH) and vasopressin (Crowe et al., 1989b) or by increasing intrinsic levels of corticosterone by isolation-induced stress (Johnston and Rose, 1998).

Facilitation of memory has also been noted with the membrane stabiliser diphenylhydantoin (DPH) (Gibbs et al., 1986; Gibbs and Ng, 1976, 1984a). DPH has been credited with several actions including effects on ion channels and synaptic transmission (Rogawski and Porter, 1990) and is also a facilitator of Na^+/K^+-ATPase activity (Guillaume et al., 1989; Gutman and Boonyaviroj, 1977; Imaizumi et al., 1995; Lampley et al., 1995; Murakami and Furui, 1994).

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Gibbs and Ng (1984a) have reported that the amnestic effects of the antibiotic cycloheximide (CXM) can be overcome by the simultaneous application of DPH. They proposed that DPH was capable of overcoming the amnesia induced by CXM by prolonging the duration of the sodium pump-dependent ITM memory phase and hence delaying the sensitivity to protein synthesis-dependent effects until the effect of the antibiotic has dissipated.

DPH has also been observed to extend the duration of the short-term memory stage and ITM (phase A) in chicks not subjected to antibiotics (Gibbs et al., 1986). Interestingly, both NA and ACTH, agents noted to be able to induce long-term memory (LTM) in weakly trained chicks and also to overcome the effects of memory inhibitors, have both been demonstrated to share a common action of facilitating Na^+/K^+-ATPase activity (Gibbs and Ng, 1976). We have also recently reported that in chicks rendered amnesic as a consequence of the application of thiamine deficiency in association with acute ethanol toxicity the memory deficit induced by the dual insult can be overcome by the application of DPH (Crowe and El Hadj, 2002).

Despite these results, an examination of memory facilitative effects of DPH, administered alone, using the WL experience has not yet been undertaken. It is also of interest to determine if the application of DPH in association with the weak training experience would result in proportionate alteration in the expression of Na^+/K^+-ATPase, the physiological process thought to underlie the development of the ITM phase of memory (Gibbs and Ng, 1977; Ng and Gibbs, 1991).

Previous research undertaken by Hájek et al. (1994) measuring Na^+/K^+-ATPase activity in 1–3-day-old chicks has indicated that enzyme activity was dramatically decreased (by between 40% and 50%) in the time interval between 10 min and 2 h after application of MeA onto the tongue of awake chicks. It should be stipulated that this stimulation did not occur in the context of normal learning and that a very large amount of MeA relative to that normally encountered in the passive avoidance learning task was applied to the tongue. It would be of more interest to investigate the responsivity of the enzyme to an actual training regime, to determine the ability of DPH to affect the activity of Na^+/K^+-ATPase activity following training with a weak training experience and to determine the neuroanatomical specificity of the activity of the enzyme in chick brain.

The aim of this series of studies was to investigate the ability of DPH to modulate memory formation in chicks trained with the weakly aversive training experience and to determine whether the application of DPH would result in consolidation of an otherwise weak training experience and to further investigate the specificity of this effect at the pharmacological level. The experimental series comprised a dose response study of DPH (10^{-7} to 10^{-4} M) using the weak training version of the passive avoidance learning task and a retention function for DPH and saline. The second aim of this study was to investigate the effects of DPH on the activity of Na^+/K^+-ATPase following training on the WL version of the task.

2. Methods

2.1. Animals

One-day-old black Australorp white Leghorn cockerels were obtained from a local hatchery on the morning of each experiment. Chicks were randomly placed in pairs into open-topped wooden boxes ($20 \times 25 \times 20$ cm). One chick in each pair was marked with a small black stripe on its head for identification purposes during data recording. Ambient temperature was maintained at 27 ± 2 °C with a 25-W clear globe suspended above each box. Chicks were left to settle in the boxes for at least 30 min before any testing procedures began. Chicks received food but not water throughout the study and this was made available by sprinkling chick mash on the floor of the pen. The yolk sac provides neonate chicks with sufficient nutrients to survive the first 5 days of life (Romanoff, 1960). For the pharmacological experiments, a group of 20 chicks constituted one experimental group. The experimental protocol was approved by the La Trobe University Animal Ethics Committee.

2.2. Passive avoidance learning task

The single trial passive avoidance learning task exploits the spontaneous tendency of chicks to peck at objects in their immediate environment. Chicks were trained using the variant of the passive avoidance training procedure described by Crowe and Hale (2002). Briefly, the task involves three components: pretraining, training and retention trials. At pretraining, chicks were presented with a chrome bead ~ 2 mm in diameter. The bead was dipped in water to encourage the chicks' natural pecking response. This procedure was repeated after a period of about 20 min. Following the second pretraining with the chrome bead, the chicks were presented with a red bead ~ 5 mm in diameter. Again, the bead was dipped in water and was then presented for 10 s. The number of pecks was recorded using a hand-held event recorder linked to a PC. The training trial involved presentation of a red bead visually identical to that used in pretraining. The bead was dipped in either 20% or 100% concentration of the chemical aversant MeA or water. Chicks typically show a disgust reaction after pecking at the aversive bead involving the chicks shaking their heads, closing their eyes and occasionally wiping their beaks on the floor of the box. The bead was presented to the chicks for 10 s. In both pharmacological and biochemical experiments, chicks that failed to peck at the training bead were excluded from the subsequent analysis. For the pharmacological experiments, the retention trial was conducted at various times following training according to the respective experimental protocols. The number of pecks at

the bead was recorded. An avoidance ratio (AR) was calculated as the number of pecks at the red pretraining bead divided by the number of pecks at the red test bead plus the number of pecks at the red pretraining bead (i.e., AR = pecks pre/pecks pre + pecks test).

For the biochemical experiment, a retention trial was not conducted. Previous studies examining reminder effects following training (Summers et al., 2000, 2003) indicate that the effect of the reminder itself (in this case, the test trial) can influence subsequent memory processing. As a result, the chicks in the current study were not subjected to a recall trial before sacrifice.

Previous research using the passive avoidance model has used a blue bead in addition to the red bead during pretraining and also during the test. It was assumed that presenting the blue bead at test provides a measure of discriminated learning (i.e., the chicks will learn to avoid the red, previously aversive bead but continue to peck freely at the blue, nonaversive bead). Recent observations in our laboratory (Crowe and Hale, 2002) indicate that chicks fail to discriminate between the red and the blue beads for a period of up to 10 min after the training trial. We believe that the use of a single bead, measured both before and after the training experience and using both MeA-trained and water-trained controls, results in a more concise characterisation of memory-related phenomenon that is not contaminated by carry-over effects from the aversive training experience to the nonaversive bead.

2.3. Drugs

DPH (Sigma-Aldrich, Castle Hill, Australia) was dissolved in sterile isotonic saline to the required concentration and injected in a volume of 100 μl/chick. It was administered by subcutaneous injection with a 27.5-gauge needle into a ventral skin fold just below the rib cage. DPH and saline were injected blindly and the codes were not broken until completion of the collection of the behavioural data.

2.4. Na^+/K^+-ATPase assay

Na^+/K^+-ATPase activity was assessed using the method described previously by Hájek et al. (1994). As diagrammatically represented in Fig. 1 the chick brain was dissected into four sections using a plastic mould and dividing the sections using a razor blade. Section 1 contained the bulbus olfactorius (BO); section 2 contained the intermediate medial hyperstriatum ventrale (IMHV) and lobus parolfactorius (LPO) as well as the hyperstriatum accessorium (HA), hyperstriatum ventrale (HV), hyperstriatum dorsale (HD) and parts of the neostriatum; section 3 contained the hippocampus, archistriatum and neostriatum caudale (NC); section 4 contained the cerebellum. Experimental groups consisted of 10 chicks for the DPH-injected samples and 5 chicks for saline-injected samples.

Chicks received injections of DPH (10^{-7} M) or saline immediately following training on the passive avoidance learning task and were decapitated at the following times after training: 10, 30, 40, 60, 180 and 1440 min. Drugs were injected blindly and the codes were not broken until after the biochemical data had been analysed.

Immediately after decapitation, the whole brain was rapidly dissected and sectioned into the four areas using a razor blade and a mould developed for this purpose (Hájek

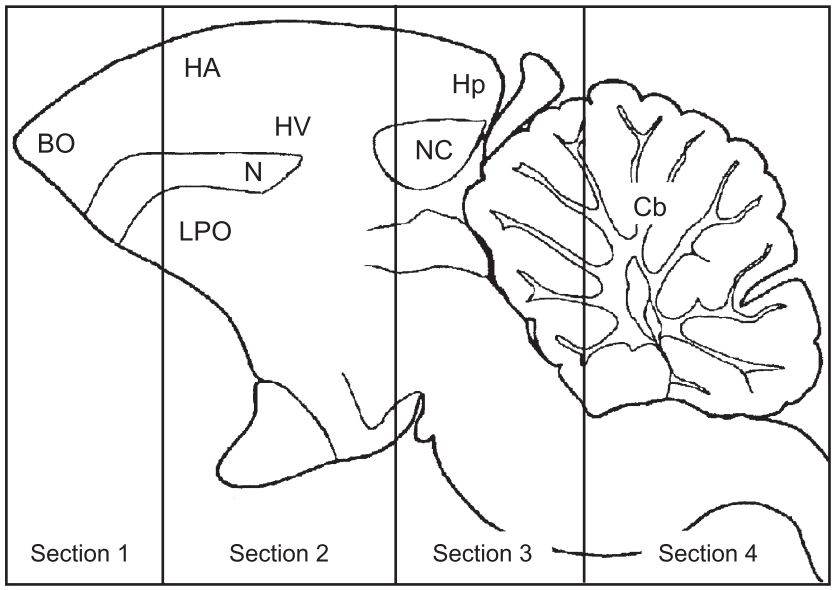

Fig. 1. Sagittal section of the chick brain (adapted from Youngren and Phillips, 1978) demonstrating the coronal sections made using the mould and razor blade. N, neostriatum; Hp, hippocampus; Cb, cerebellum.

et al., 1994). Tissues were transferred into 2 ml precooled 50 mM Tris-HCl buffer (pH 7.4) within 40 s and homogenized with a Teflon glass homogenizer at 4 °C. Samples were centrifuged twice at 3000 rpm for 20 min at 4 °C and the supernatant was collected and stored at 4 °C. On the next day, the supernatant was transferred into 1.5 ml Eppendorf tubes and centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant was saved and stored at − 20 °C until the assay was performed.

Na^+/K^+-ATPase activity was estimated using ouabain (1 mM) as a specific inhibitor. ATPase activity was assayed in an incubation mixture that contained NaCl 67 mM, KCl 2 mM, $MgCl_2$ 3 mM, and Tris-HCl 10 mM (pH 7.8). The protein concentration of samples was determined using a BSA standard at $A_{280\ nm}$ by spectrophotometer. Twenty microlitres of membrane suspension containing 20–30 μg of protein were added to the incubation mixture. Samples were preincubated for 15 min and the reaction was started by the addition of ATP (vanadium-free) buffered with Tris to a final concentration of 1.3 mM. All incubations were carried out for 10 min at 37 °C, and the reaction was stopped by the addition of 20% trichloroacetic acid. The resulting inorganic phosphate (P_i) was then determined by adding Taussky and Shorr (1953) reagent and reading the results at $A_{655\ nm}$ using a Benchmark microplate reader (Bio-Rad, Regents Park, Australia).

3. Results

3.1. Experiment 1: effects of DPH on memory using a weak (20% MeA) training stimulus

The first experiment undertook a dose response study with DPH (10^{-7} to 10^{-4} M) and saline administered immediately after 20% aversant training on the passive avoidance task. These doses were selected from a previous study conducted with DPH and strong MeA training (Gibbs and Ng, 1976), which indicated that doses in this range were effective in facilitating recall of training, while lower or higher doses did not. Chicks were tested for retention at 180 min. A one-way ANOVA was conducted on the data. The results indicated a significant effect for Drug [$F(4,89)=3.66$, $P=.008$, $\eta^2=0.141$]. These data are presented in Fig. 2.

Post hoc Dunnett's tests between saline-treated and DPH-treated chicks on the weak training version of the task indicated memory facilitation with each dose of DPH (10^{-7} M, $P=.014$; 10^{-6} M, $P=.013$; 10^{-5} M, $P=.001$; 10^{-4} M, $P=.016$). The lowest dose (10^{-7} M) was thus used for the subsequent experiments.

3.2. Experiment 2: retention function with DPH and saline using a weak (20% MeA) training stimulus

The first experiment indicated that administration of doses of 10^{-7} to 10^{-4} M of DPH immediately after training facilitated memory for a weak training stimulus. The aim of experiment 2 was to characterise the retention function for DPH and saline with chicks trained with a weak (20% MeA) version of the task.

Chicks were trained with either 20% MeA or water and injected with either 10^{-7} M DPH or saline. Retention was tested at 10, 20, 30, 40, 60, 180 and 1440 min post-training. A three-way ANOVA [Drug (2) × Training (2) × Training test interval (7)] detected a significant effect for Drug [$F(1,517)=8.448$, $P=.004$, $\eta^2=0.016$], Training [$F(1,517)=425.892$, $P<.001$, $\eta^2=0.452$] and Training test interval [$F(6,257)=2.891$, $P=.01$, $\eta^2=0.041$]. There were no significant interaction effects. These data are presented in Fig. 3. The significant training effect suggests that drug-treated chicks trained with water continued to freely peck at the retention bead at test. Therefore, the avoidance observed after MeA training represents a memory effect rather than a more generalised effect on motor functioning or motivational factors.

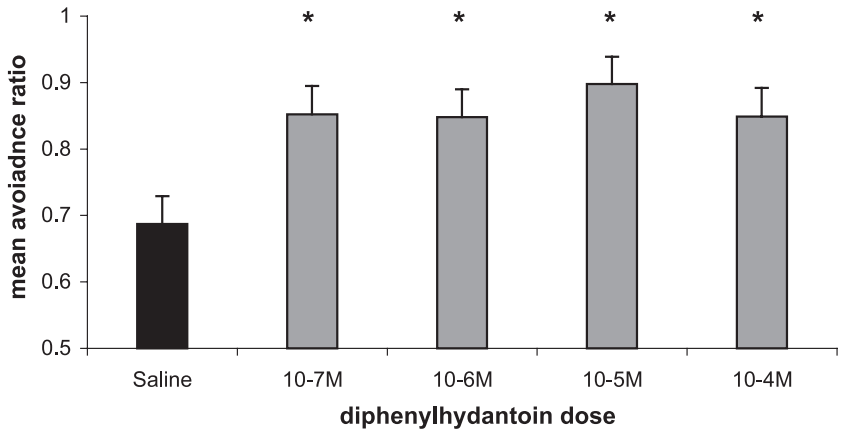

Fig. 2. The effect of DPH on retention for weak (20% MeA) training calculated as an AR (+S.E.M.). Chicks were injected immediately after training and tested for retention after 180 min (*$P<.05$, comparison between DPH and saline).

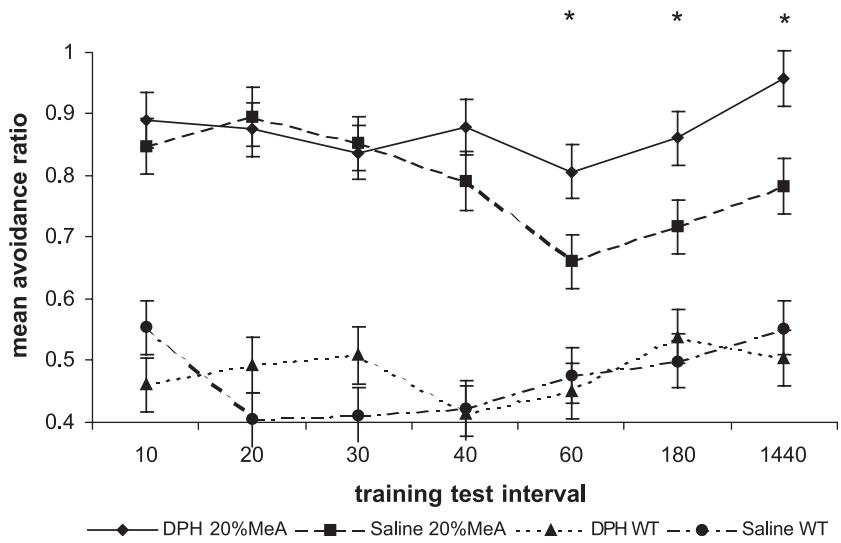

Fig. 3. The effect of DPH (10^{-7} M) on retention of a weak training stimulus at various times post-training. Calculated as an AR (± S.E.M.). Chicks were injected immediately after training (* $P<.05$, comparison between aversant-trained chicks; WT, water).

Pairwise analysis for the MeA-trained birds revealed significant differences ($\alpha<.05$) between the saline-treated and the drug-treated groups at 60 and 180 min and 24 h after training (see Fig. 3). This indicates that the 20% training when paired with a 10^{-7} M dose of DPH immediately following the training experience leads to consolidation of the training into LTM.

3.3. Experiment 3: effects of DPH on Na^+/K^+-ATPase activity in the chick brain after a weak (20% MeA) training experience

Fig. 4 shows the mean Na^+/K^+-ATPase activity (μmol P_i/mg protein/min) in the whole chick brain after passive avoidance training with DPH-treated and saline-treated chicks trained with a weak (20%) MeA aversant. A three-way ANOVA [Drug (2) × Time (7) × Section (4)] revealed a significant main effect for Drug [$F(1,362)=89.113$, $P<.001$, $\eta^2=0.198$], Time [$F(6,362)=21.106$, $P<.001$, $\eta^2=0.259$], Section [$F(3,362)=139.625$, $P<.001$, $\eta^2=0.536$], Drug × Time [$F(6,362)=3.299$, $P=.004$, $\eta^2=0.052$] and Drug × Section [$F(3,362)=5.618$, $P=.001$, $\eta^2=0.044$].

To determine the specificity of the effect of DPH on Na^+/K^+-ATPase activity, post hoc independent-samples t tests were performed on DPH-treated and saline-treated chicks trained on 20% MeA. This analysis indicated significant differences at 10 min [$t(58)=3.191$, $P=.002$], 20 min [$t(58)=3.404$, $P=.001$], 30 min [$t(58)=2.521$, $P=.014$] and 1440 min [$t(58)=3.175$, $P=.002$] after training. The difference between DPH-treated and saline-treated chicks approached significance at 60 min [$t(58)=1.940$, $P=.057$].

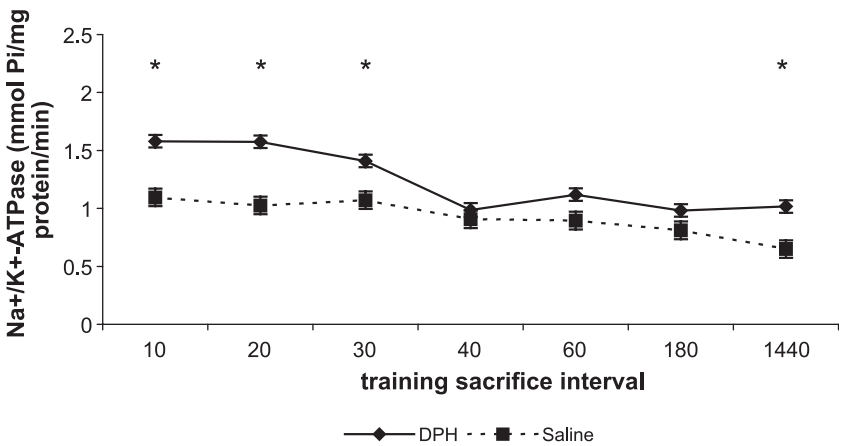

Fig. 4. The effect of DPH (10^{-7} M) on Na^+/K^+-ATPase activity (± S.E.M.) in the whole brain of chicks trained on the weak version of the passive avoidance task (* $P<.05$).

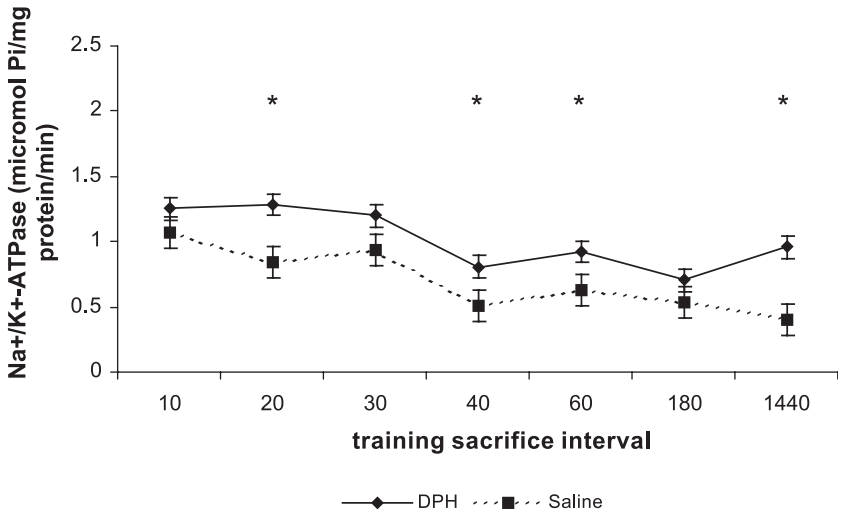

Fig. 5. The effect of DPH (10^{-7} M) on Na^+/K^+-ATPase activity (± S.E.M.) in section 1 of the brain of chicks trained on the weak (20% MeA) version of the passive avoidance task (* $P<.05$).

To better understand the Section main effect and Drug × Section interaction, each section was then analysed separately.

DPH facilitated Na^+/K^+-ATPase in section 1 of the chick brain (see Fig. 5). This section contained part of the forebrain including the BO. A two-way ANOVA [Drug (2) × Time (7)] revealed a main effect for Drug [$F(1,91)=31.634$, $P<.001$, $\eta^2=0.258$] and Time [$F(6,91)=9.510$, $P<.001$, $\eta^2=0.385$] but no interaction effect [$F(6,91)=0.876$, $P=.516$, $\eta^2=0.055$].

To determine the time at which DPH facilitated Na^+/K^+-ATPase, post hoc independent-samples t tests were performed on DPH-treated and saline-treated chicks trained on 20% MeA. The results indicated significant differences between DPH-treated and saline-treated chicks at 20 min [$t(13)=3.015$, $P=.01$], 40 min [$t(13)=2.632$, $P=.021$], 60 min [$t(13)=3.254$, $P=.006$] and 1440 min [$t(13)=5.954$, $P<.001$] after training.

The second section of the chick brain (see Fig. 6) contained several structures known to be critical to passive avoidance learning, including the IMHV, HA, HD and LPO. A two-way ANOVA [Drug (2) × Time (7)] detected a main effect for Drug [$F(1,91)=19.953$, $P<.001$, $\eta^2=0.180$] and Time [$F(6,91)=6.762$, $P<.001$, $\eta^2=0.380$]. The interaction was not significant [$F(6,91)=0.271$, $P=.115$, $\eta^2=0.104$]. Independent-samples t tests were performed on

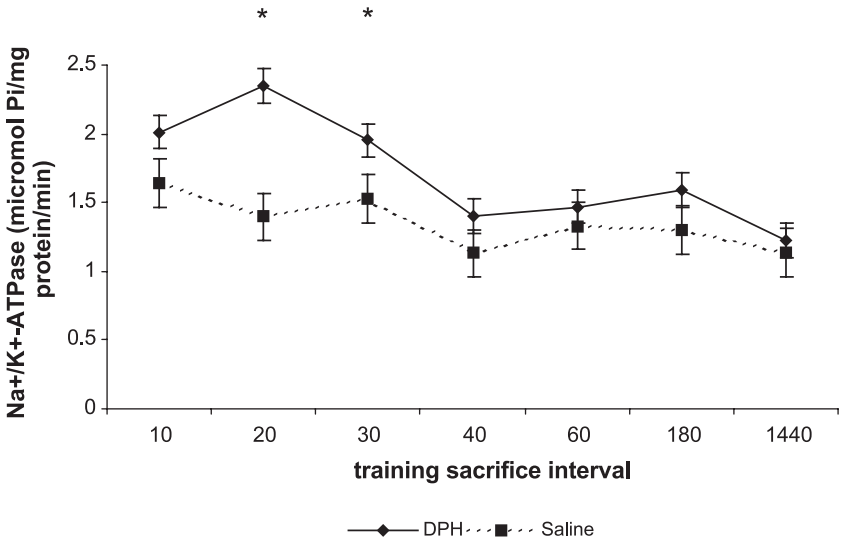

Fig. 6. The effect of DPH (10^{-7} M) on Na^+/K^+-ATPase activity (± S.E.M.) in section 2 of the brain of chicks trained on the weak (20% MeA) version of the passive avoidance task (* $P<.05$).

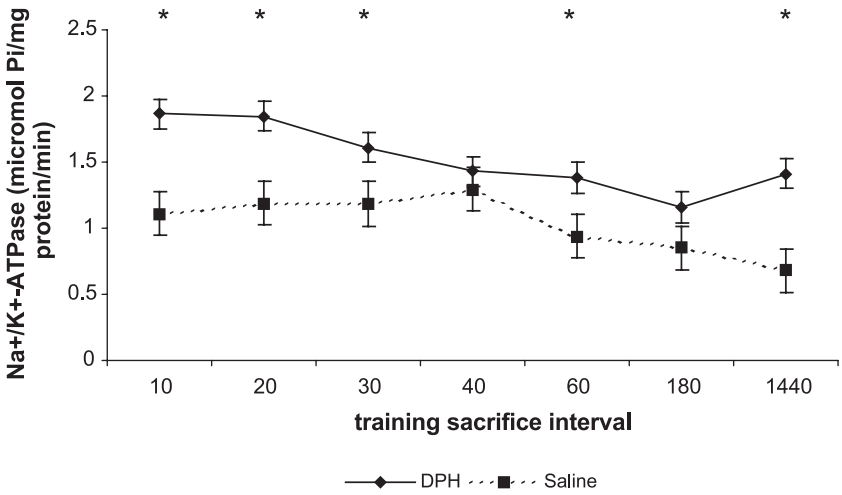

Fig. 7. The effect of DPH (10^{-7} M) on Na^+/K^+-ATPase activity (± S.E.M.) in section 3 of the brain of chicks trained on the weak (20% MeA) version of the passive avoidance task (* $P<.05$).

DPH-treated and saline-treated chicks. The results indicated significant effects at 20 min [$t(13)=6.696$, $P<.001$] and 30 min [$t(13)=2.832$, $P=.014$] after training and not at the other times sampled.

The third section of the chick brain (see Fig. 7) contained structures known to be important for passive avoidance learning including the hippocampus, archistriatum and NC. A two-way ANOVA [Drug (2) × Time (7)] detected a main effect for Drug [$F(1,91)=41.943$, $P<.001$, $\eta^2=0.315$] and Time [$F(6,91)=4.301$, $P=.001$, $\eta^2=0.221$]. The interaction effect did not reach significance [$F(6,91)=0.178$, $P=.259$, $\eta^2=0.080$]. To determine the time at which DPH facilitated Na^+/K^+-ATPase activity, post hoc independent-samples t tests were performed on the DPH-treated and saline-treated chicks. The results indicated significant differences at 10 min [$t(13)=2.923$, $P=.012$], 20 min [$t(13)=2.603$, $P=.022$], 30 min [$t(13)=2.313$, $P=.038$], 60 min [$t(13)=2.251$, $P=.042$] and 1440 [$t(13)=3.983$, $P=.002$] after training.

Section 4 of the chick brain contained the cerebellum. A two-way ANOVA [Drug (2) × Time (7)] detected a significant effect for Drug [$F(1,89)=4.164$, $P=.044$, $\eta^2=0.045$] and Time [$F(6,89)=5.133$, $P<.001$, $\eta^2=0.256$]. The Drug × Time interaction was also significant [$F(6,89)=4.119$, $P=.001$, $\eta^2=0.217$]. Post hoc independent-samples t tests were performed on DPH-treated and saline-treated chicks trained on 20% MeA. The results indicated significant differences at 10 min [$t(13)=3.420$, $P=.005$] and 40 min [$t(13)=-3.192$, $P=.002$] after training (see Fig. 8).

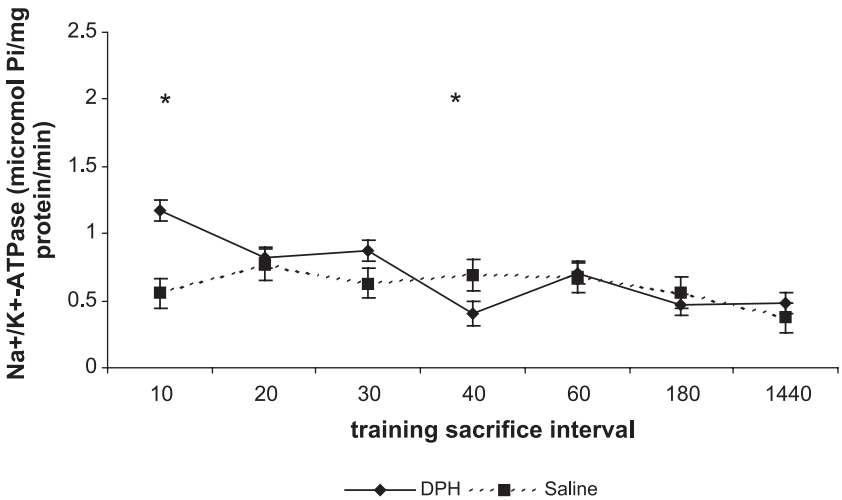

Fig. 8. The effect of DPH (10^{-7} M) on Na^+/K^+-ATPase activity (± S.E.M.) in section 4 of the brain of chicks trained on the weak (20% MeA) version of the passive avoidance task (* $P<.05$).

4. General discussion

The results of the pharmacological experiments presented in this series indicate that (1) memory following a weak training experience can be facilitated using DPH, (2) this facilitation can take place with a range of doses of DPH spanning from 10^{-7} to 10^{-4} M and (3) the pairing of the weak training experience with DPH results in facilitation of memory, which can be observed from 40 min following training.

Previous studies conducted in Gibbs and Ng's laboratories have indicated a consistent series of results, which implicate Na^+/K^+-ATPase activity in the processing of memory. DPH given immediately after learning extends the duration of the susceptibility of LTM to CXM inhibition, with CXM administered 30–40 min after learning still inducing amnesia in DPH-pretreated chicks but not those treated with saline. Following an injection of DPH immediately after learning, a second injection of DPH given at appropriate times after CXM treatment at 10 or 30 min after learning prevents amnesia (Gibbs and Ng, 1984b).

The results of this series of studies provide clear support for the involvement of Na^+/K^+-ATPase in memory formation following both weak and strong aversant training experiences. The results thus support the previous observations of Gibbs and Ng (1976, 1978, 1984a) regarding the role of Na^+/K^+-ATPase in memory processing.

The results from the biochemical experiment indicate that (1) for the whole brain, differences were observed between saline-treated and DPH-treated chicks at 10, 20, 30 and 24 h after the weak training. (2) In section 1, which included the BO, higher activity was observed at 20, 40, 60 and 24 h after the training. (3) Section 2 contained the IMHV and LPO as well as the HA, HV and HD, and parts of the neostriatum were only noted to feature difference at 20 and 30 min following training. These are the times thought to coincide with the development of the ITM stage in the Gibbs and Ng model of memory. (4) Section 3, which contained the hippocampus, archistriatum, and NC, showed differences at all times measured with the exception of 40 min. (5) Section 4, which contained the cerebellum only, showed differences at 10 and 40 min post-training. It was only at the 40-min time point in section 4 that the level of activity of the saline-treated chicks was elevated above that of the DPH-treated ones.

These data clearly demonstrate that DPH facilitates the activity of Na^+/K^+-ATPase at the majority of times sampled in a large percentage of the sections of the chick brain. The most marked level of elevation in the activity of the enzyme was observed at the 20-min time point following weak training in section 2. This represents a 68% increase in the activity of the enzyme in those areas considered to be crucial to the processing of memory in the paradigm at a time predicted by previous investigation (Gibbs and Ng, 1977, 1984b; Ng and Gibbs, 1991) to be crucial in the development of the ITM stage of memory.

In the study conducted by Hájek et al. (1994), the level of enzyme activity was noted to decrease by as much as 40–50% in the whole brain by 10 min following the application of the aversant to the tongue. Unfortunately, it was not possible to directly compare the data between this study and that of Hájek et al. (1994).

In the Hájek et al. (1994) study, the chicks were subjected to administration of a bolus of the aversant applied to the beak and were not involved in a learning trail as such. Thus, there were considerable differences with both the intensity of the stimulus [i.e., strong (100% MeA) versus weak (20% MeA) aversant] and the volume of the aversant [i.e., high (50 μl) versus low (probably at most 10 μl) per chick] to which the chicks were exposed. In addition, the manner in which Hájek et al. (1994) prepared their sections differed from the current study in that we used the whole slice as described in Section 2, whereas Hájek et al. (1994) undertook further dissections within each slice. This latter measure seemed to add the complication of inconsistency of neuroanatomical technique, possibly compounding the level of experimenter artifact, and thus was not adopted in the current study.

Another interesting aspect of the Hájek et al. (1994) study was that they noted that the application of the aversant produced a marked decrement (i.e., 40–50% decrease) in enzyme activity. These investigators speculated that this might mean that substances with inhibitory effects may be released after MeA administration that bind to specific receptors in the membrane fraction. While this may well be true, in our study, the level of enzyme was noted to increase (by as much as 68% at 20 min following weak training in the section containing several memory relevant neural structures) as a consequence of the application of DPH and resulted in consolidation of the otherwise weak training experience. Clearly, considerably more study needs to be undertaken into exactly how the facilitation or inhibition of the enzyme occurs in the context of the actual training experience and how these effects impact on the physiological and biochemical processing of the neural event.

DPH has been credited with several actions including direct effects on ion channels and synaptic transmission (Rogawski and Porter, 1990); decreasing the membrane resistance with little or no change in the resting membrane potential (Ayala et al., 1977); effects on monoamines, glutamate, and GABA activity (Cunningham et al., 2000; Okada et al., 1997) and effects on folate activity (Carl et al., 1997). In addition to these other actions, it is also a known facilitator of Na^+/K^+-ATPase activity (Guillaume et al., 1989, Gutman and Boonyaviroj, 1977; Imaizumi et al., 1995; Lampley et al., 1995; Murakami and Furui, 1994) particularly in situations of cerebral ischaemia. Gibbs and Ng (1984a) have proposed that DPH was capable of overcoming the amnesia induced by CXM by prolonging the duration of the sodium pump-dependent ITM memory phase and hence delaying the sensitivity to protein synthesis-dependent effects until the effect of the antibiotic has dissipated.

These observations are also consistent with the wider literature regarding the role of Na^+/K^+-ATPase in memory processing in species other than the chick. The investigation of Ross and Soltesz (2000) clearly indicates a role for the electrogenic sodium pump in setting the level of excitability of hippocampal interneuronal networks after traumatic injuries such as closed head injury. The study of Plataras et al. (2003) supports the notion that the memory facilitatory effects of cytidinediphosphocholine with aged rats occur as a consequence of the ability of the agent to restore hippocampal acetylcholinesterase and Na^+/K^+-ATPase in these animals, indicating the important role that these enzymes play in memory processing and as a target for rehabilitation strategies in memory impairment.

A possible mechanism of how the observed effects of Na^+/K^+-ATPase on memory processing noted in the current study could take place may be provided by the work of Dzhandzhugazyan and Bock (1997). These investigators have indicated that the neural cell adhesion molecule (NCAM) contains an ATP binding site, which is localized extracellularly and is probably catalytic in function. Binding the substrate or fluorosulfonylbenzoyl adenosine protected a proteolytic cleavage site in NCAM localized close to the membrane, presumably by induction of a local conformational change in NCAM, indicating a mechanism by which ATP may regulate NCAM adhesion and adhesion-triggered processes. These authors contend that this finding may indicate a possible role of this mechanism in synaptic plasticity and memory consolidation, consistent with the results noted in the current series of studies.

The findings of the current study have not specifically explored the role of lateralised biochemical activity in the consolidation of the weak training experience (Gibbs et al., 2003). This presents an exciting further development of the issues raised by the current study, which are currently under investigation in our laboratory.

The results of this series of studies support the notion that Na^+/K^+-ATPase plays an important role in memory processing following passive avoidance training in the 1-day-old chick. This role is illustrated by facilitation of the memory processing using DPH, which results in long-term recall of a weak training event. This role is further illustrated by facilitation of the enzyme using DPH in the context of a weak training regime and establishing that weak training in association with DPH results in facilitation of Na^+/K^+-ATPase activity in several sections of the chick brain and at a time thought to be important to the development of the ITM stage of memory.

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