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The foraging gene affects adult but not larval olfactory-related behavior in *Drosophila melanogaster*

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Abstract

This study investigates the ability of larvae and adult rover and sitter *Drosophila melanogaster* to detect and migrate towards the source of a fly medium attractant using larval plate assays and an adult olfactory trap assay. Allelic variation at the foraging locus which encodes a cGMP-dependent protein kinase (PKG) did not affect larval olfactory response in the larval plate assays. In contrast, adult males of the sitter mutant for^{s2} exhibited an olfactory trap response (OTR) which was significantly greater than that of males of the wild type for^{R} strain from which for^{s2} was derived and further genetic analysis showed that this was attributable to the for^{s2} allele. The olfactory responses of for^{R} and for^{s2} flies to three odours (propionic acid, ethyl acetate and acetone) in a T-maze assay was normal indicating that they did not have general olfactory deficits. The finding that adult flies who differ in their PKG enzyme activities differ in foraging behaviours and olfactory trap responses to yeast odours suggests that PKG signalling pathways are involved in olfactory related responses to food.

Keywords: Olfaction; Foraging; Behavior; Genetics; *Drosophila*

1. Introduction

The senses are the means by which organisms obtain information regarding their surroundings. *Drosophila* are sensitive to a wide variety of odourants and are capable of odour discrimination. The third antennal segment and maxillary palp of the adult and the dorsal organ of the larval antenno–maxilary complex have been identified as the main olfactory organs of this organism [2,5,35,36]. The underlying signal transduction processes which mediate the olfactory avoidance, attraction and adaptation responses which result when an odour is detected by these olfactory organs are not well defined. However, mutagenesis experiments have begun to identify a number of genes which mediate the manner and/or rate with which *Drosophila melanogaster* respond to various odours (olfactory response). The products of several of these genes have been characterized and have been found to play a role in a number of signal transduction pathways (e.g. [13,20,24,27,38,43]).

The natural habitat of *Drosophila* is a complex one which is continually changing. Adult females of many species deposit eggs on substrates (e.g. fruit) [6] which contain microorganisms such as yeasts, which metabolically alter these substrates and release products such as alcohols, acids, esters, ketones and aldehydes [6,22]. Thus, while foraging for food, *Drosophila* are exposed to an abundance of olfactory stimuli. It is likely that olfactory response plays an important role in the selection of food [3] and in the identification of hazardous substances [21].

D. melanogaster larvae forage for food by shovelling with their mouth hooks while moving along the surface of the feeding substrate [23,37]. This foraging behaviour is measured as the distance a larva travels on a nutritive

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yeast and water substrate. As the larva moves along the feeding substrate it leaves a visible trail. The length of this trail, produced in a 5-min interval, is called the path length [37]. Adult flies also exhibit stereotyped foraging behaviours. Once a fly has fed on a resource it pivots around it, turning many times. After a period of this 'intensive search' behaviour, the fly walks in a relatively straight line away from the resource—a behaviour called 'ranging' [7,8,28].

Allelic variation at the foraging (*for*) gene alters the amount of locomotion during foraging in both larval and adult *D. melanogaster* [17,19,32,37]. Larvae with the rover allele, for^R, have long foraging path lengths while those homozygous for the sitter allele for^s have significantly shorter trails [37]. These foraging phenotypes are maintained through metamorphosis to the adult stage with adults homozygous for rover alleles walking further from a sucrose drop post feeding than those homozygous for sitter alleles [32]. Genetic analysis has shown that the rover allele is completely dominant to the sitter allele [18,37] and that the region to which *for* has been localized on the polytene chromosome map (24A3-5) corresponds to that of the gene *dg2* [30].

The gene *dg2* is one of two *D. melanogaster* genes encoding cGMP-dependent protein kinase (PKG) and is transcribed throughout development into three major mRNA species and several minor transcripts [25]. The cloning of *for* revealed that it was synonymous with *dg2* [30]. Flies homozygous for the wild type sitter (for^s) allele and all sitter mutants have reduced PKG activity in enzyme assays relative to flies homozygous for the wild type rover (for^R) allele [30].

The biological function of PKG in the nervous system and its role in signal transduction pathways have remained elusive for a variety of reasons. These include: the low levels of PKG found in most tissues; the observation that PKG is not involved in all intracellular cGMP signalling [31]; the phenotypes that this enzyme might influence were unknown and the isolation of mutations in genes coding for PKG had been unsuccessful. However, cGMP signalling pathways have been implicated in the olfactory response of both vertebrates and invertebrates (e.g. [4,11,12,29]).

It may be that differences in PKG enzyme activity are manifest in flies as differences in ability to perceive or respond to about the environment during foraging. In addition to exhibiting contrasting foraging behaviours, flies with different levels of PKG activity may also differ in the manner and/or rate with which they respond to a nutritive fly food attractant. To investigate this possibility, the ability of larvae and adult rover and sitter *D. melanogaster* to detect and migrate towards the source of a fly medium attractant was evaluated using larval assays and the olfactory trap assay, respectively.

2. Materials and methods

2.1. Fly strains

The for^{s2}/for^{s2} is a viable sitter-behaving mutant strain produced by γ-mutagenesis of rover behaving for^R/for^R flies; $for^{l(92)}$ is a pupal lethal sitter-behaving mutant strain which is maintained as a balanced heterozygote with *In(2LR), Cy* dp^{lvI} *pr* cn^2, hereafter referred to as *CyO* [19]. The *Df(2L)* ed^{Sz}/CyO strain carries a deficiency (24A03-04;24D03-04) which uncovers the *for* locus [19] and was obtained from the Bloomington Stock Center. All flies were housed in bottles containing standard fly medium at $25 \pm 1°$ on a 12L:12D light cycle with lights on at 08:00 h (standard conditions).

2.2. Larval olfaction

To measure larval olfactory response two plate assays were used. The large plate assay was modified from [42]. A total of 50 larvae were placed in a large petri dish (150 mm diameter) containing 25 ml of 3.2% agarose. A small petri dish (60 mm diameter) containing 10 ml of food medium was placed in the center of the large dish. No lid was placed on the smaller dish, so that larvae capable of detecting the food odour would crawl up and over the edge of this dish to reach the attractant. After 30 min the number of larvae which did not enter the small dish was noted. A response index (RI) ranging from 100 (total attraction) to -100 (total repulsion) was calculated for each dish ($RI = [(n^{food} - n^{agar})/total] \times 100$) [14]. The second plate assay was modified from that described by [1]. The assay plate was a plastic petri dish (8.5 cm diameter, 1.4 cm height) containing 10 ml of 3.2% agar. An agar plug (1.5 ml of 1.6% agar) and a food attractant plug (1.5 ml of standard fly medium) were placed diametrically opposed to each other at the edges of the plate. A total of 20 3rd instar larvae (95 ± 1.5 h old) were placed in the center of the plates, the cover was replaced and after 10 min the number of larvae on either side of the dish was noted. The position of the food plug and agar plug were alternated between dishes. A RI was calculated for each dish as described above. The mean RI for each strain and assay were calculated and the data were statistically analysed as described below.

2.3. Olfactory trap assay

The olfactory trap assay tests the ability of adult flies to detect and migrate towards the source of an olfactory attractant. This study utilized a modified version of the olfactory trap protocol described by [42]. Traps were constructed from a microfuge tube (1.5 ml) and two (1–200 μl) micropipette tips. The tip of the mi-

crofuge tube was removed approximately 2.5 mm from its end using a razor blade. The narrow end of each micropipette tip was removed by cutting 1 cm from the terminus (creating an opening 1 mm in diameter). One micropipette tip was cut again approximately 1.6 cm from the long end. The small end of this 'double cut' tip was inserted into the microfuge tube and the larger tip was inserted snugly over the 'double cut' tip with its small end outside. A 1 ml pellet of solid fly medium (a dead-yeast–agar–sucrose medium) was placed inside the microfuge tube near the capped end. In this assay flies can only reach the odour by passing through the small opening in the micropipette tip at the end of the trap.

The pipette tip with the small end inside the microfuge tube makes it difficult for a fly to exit the trap. Each trap was placed in a Petri dish (8.5 cm diameter, 1.4 cm height) containing 10 ml of 1.6% agarose. For collection, flies were placed on ice and ten 1–4 day old male flies from a given strain were placed into each petri dish containing a trap. The trap dishes were randomly divided between several boxes and kept at 21 ± 1°C. The boxes were closed to maintain darkness for the duration of the test period and opened only to count the number of flies in the traps. A dish containing water was placed in each box to maintain humidity. A minimum of 18 dishes with traps were set up for each strain tested. The number of flies in each trap (olfactory trap response) (OTR) was determined after 12, 24, 48 and 64 h. Two to five control trap dishes containing no odourant were run for each strain for each test. OTR did not differ between the strains when yeast was not included in the medium (data not shown). OTRs from the 48-h time point from trap dishes containing a minimum of nine out of ten surviving flies were statistically analyzed. Preliminary experiments revealed that while the mean number of trapped flies increased over time (i.e. 0–64 h) the number of surviving flies decreased (data not shown). Hence, the 48-h time period was chosen for subsequent experiments since after this time the rate of death increased dramatically and greatly decreased sample sizes.

2.4. Adult size measurements

To determine if any differences in the OTRs of homozygous for^{s2} and for^{R} flies were due to differences in body sizes between the strains the wing length (to obtain an estimate of fly length) and the distance between the wings (to obtain a width measurement) of 1–4-day-old for^{s2} and for^{R} males were measured using a Zeiss WILD light microscope. These data were statistically analyzed as described below.

2.5. T-maze assay

To assess the odour specificity of the OTRs of for^{R} and for^{s2} flies the odour avoidance responses of these flies were tested using the T-maze assay modified from [40] and provided by [16]. Groups of approximately 100 2–4-day-old flies were aspirated into an acclimation tube through which air was drawn (750 ml/min) for 130 s. The flies were subsequently tapped into an elevator-like chamber which was used to deliver them to the choice point of the T-maze. At this location the flies were exposed to two currents of air (1500 ml/min). One of the currents drew an odour (S+) from the end of one of the arms of the T-maze while the other current carried fresh air from the end of the opposite arm. The flies were given 120 s to choose between the odour and the air. After this time had elapsed, the elevator was raised, the flies were trapped inside the arms of the T-maze, anaesthetized and counted.

The olfactory responses of for^{R} and for^{s2} homozygotes to an organic acid, e.g. propionic acid (pure, 0.1 dilution, 0.01 dilution), a ketone, e.g. acetone (pure, 0.01 dilution), and an acetate ester, e.g. ethyl acetate (pure, 0.01 dilution), were assessed. Odourants were diluted in heavy mineral oil (Fisher). The odour and air sources were alternated between the left and right arms of the T-maze to correct for side biases. All tests were run at 21–23°C in dim red light (approx. 665 nm).

For each group of flies tested, a normalized performance index (PI) [10] was calculated using the equation below Eq. (1):

$$\mathrm{PI} = \left[\left[\frac{(\#\text{ flies in S+ arm})}{(\#\text{ flies in S+ arm}) + (\#\text{ flies in air arm})} - 0.5\right]\Big/ 0.5\right] \times 100 \quad (1)$$

This index yields scores ranging from '0' (no avoidance) to '100' (total avoidance) [30]. The mean PI for each strain and odour concentration was calculated and the data were statistically analyzed as described below.

2.6. Statistics

All data were analyzed using one-way analyses of variance (ANOVA). If variances were not homogenous the data were transformed. To determine which strains exhibited significantly different olfactory responses, the Student–Newman–Keuls test was performed as an a posteriori test.

Table 1
Larval olfactory response to a fly food attractant[a]

Strain	Response index	
	Large dish assay	Small dish assay
for^{s2}/for^{s2}	82.0 ± 5.8 (9)	44.7 ± 12.8 (8)
for^{R}/for^{R}	82.0 ± 5.2 (8)	59.2 ± 11.3 (6)

[a] Mean response indices (RI) are reported ± S.E. with number of assay dishes in parenthesis. The mean RIs of for^{s2} and for^{R} larvae were not significantly different in either assay (one-way ANOVA, $P > 0.05$).

3. Results

3.1. Larval olfactory response

The olfactory responses of homozygous for^{R} and homozygous for^{s2} larvae to the food attractant were not significantly different in both the large and small dish assays (Table 1).

3.2. Adult olfactory trap response

The number of flies in the olfactory trap assay is an indication of the ability of the flies to detect and migrate towards the source of an odour [42]. That is, the higher the number of flies in the trap the greater the ability. Fig. 1 shows that on average significantly more

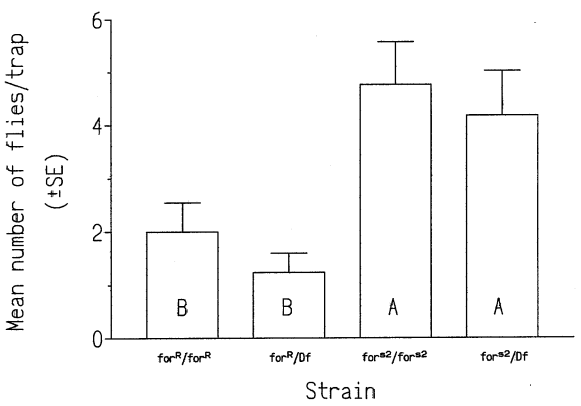

Fig. 1. Olfactory trap responses of homozygous and hemizygous for^{R} and for^{s2} flies. Mean number of flies in the olfactory traps containing a food medium attractant after 48 h. The number of homozygous for^{s2} flies entering the traps after 48 h was significantly greater than the number of homozygous for^{R} *flies*. The increased olfactory trap response of for^{s2} is not rescued by the deficiency $Df(2L)ed^{Sz}$(Df). The number of for^{s2} hemizygous flies entering the traps did not differ significantly from that of for^{s2} homozygotes, but was significantly higher than the number of for^{R} flies entering olfactory traps (one-way ANOVA calculated on square root transformed data, $F_{(3,63)} = 5.54$, $P \leq 0.002$, $16 \leq n \leq 17$). Means are presented ± S.E. Different letters represent different SNK mean groupings.

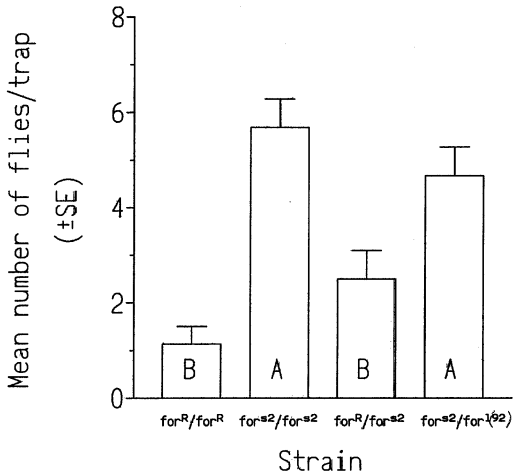

Fig. 2. Tests for complementation between for^{s2} and $for^{l(92)}$. The mean number of for^{R}/for^{s2} heterozygotes entering the olfactory traps in response to the food medium attractant was not significantly different from the number of homozygous for^{R} flies entering the traps but was significantly lower than the number of for^{s2} homozygous flies entering the traps (one-way ANOVA, $F_{(3,81)} = 13.17$, $P \leq 0.0001$, $15 \leq n \leq 18$). The number of flies heterozygous for the for^{s2} and $for^{l(92)}$ alleles entering the traps was not significantly different from that of for^{s2} homozygous flies. Means are presented ± standard error. Different letters represent different SNK mean groupings.

for^{s2} flies than for^{R} flies were found in food baited traps at the end of the 48 h test period. The deficiency $Df(2L)ed^{Sz}$ (*Df*) uncovers *for* gene function [19]. The OTR of for^{s2} hemizygotes did not differ significantly from for^{s2} homozygotes, but was significantly higher than for^{R} homozygotes (Fig. 1). Therefore, the increased olfactory trap response (IOR) phenotype of for^{s2} maps withing the deficiency that uncovers *for*.

Tests for complementation between for^{s2} and other *for* alleles suggest that the IOR of for^{s2} is associated with the *for* locus. The OTR of for^{R}/for^{s2} heterozygotes did not differ significantly from that of the homozygous for^{R} flies and was significantly lower than that of the homozygous for^{s2} flies. Thus the IOR of the for^{s2} allele is recessive to that of the for^{R} allele. The lack complementation as measured in the olfactory trap assay between the for^{s2} allele and the pupal lethal sitter allele $for^{l(92)}$ demonstrates that the IOR truly maps to the *for* locus rather than to a closely linked gene falling within $Df(2L)ed^{Sz}$ (Fig. 2). This complementation pattern suggests that the mutations in *dg2* (*for*) affect adult olfactory trap response.

The possibility that flies exited from the traps and re-entered could not be eliminated since our observation of the traps was not continuous. However, in only three of 300 observations was the number of flies inside the trap less than that of the number at the previous observation. Hence, the rate of exit from the traps was considered to be insignificant. Similarly, very few flies entered the food baited traps by chance. This was

evidenced by high signal-to-noise ratios of our assays where signal and noise are defined as the fraction of animals responding in the presence and absence of the odourant respectively. This ratio compares the number of flies entering the trap containing odour to the number entering in the absence of an odourant [42]. A typical ratio from our experiments was 11.5. Width and wing length measurements of the for^{s2} and for^{R} homozygotes did not differ significantly: $\bar{x}_{width}for^{s2} = 0.816 \pm 0.013$ mm ($n = 20$); $\bar{x}_{length}for^{R} = 0.816 \pm 0.009$ mm ($n = 20$); $\bar{x}_{length}for^{s2} = 2.12 \pm 0.024$ mm ($n = 20$); $\bar{x}_{length}for^{R} = 2.16 \pm 0.012$ mm ($n = 20$) (one-way ANOVA; $P \geq 1.00$). Therefore, the observed differences in OTRs of homozygous for^{R} and for^{s2} flies were not a function of differences in body size.

3.3. T-maze assay response

Fig. 3 depicts the olfactory avoidance responses of the for^{R} and for^{s2} flies to three different odours. The performance indices (PIs) of the for^{R} and for^{s2} adults for propionic acid, ethyl acetate and acetone did not differ significantly.

4. Discussion

The results of this study provide genetic evidence that PKG activity levels influence adult olfactory trap response to a food medium attractant. The γ-induced for^{s2} lesion in the 5′ region of the *for* locus affects adult olfactory trap response to a food medium attractant. Specifically, the OTR of adult male flies homozygous for the for^{s2} allele was significantly greater than that of flies homozygous for the wild type for^{R} allele. Complementation tests with the wild type for^{R} allele and a pupal lethal allele of *for* ($for^{l(92)}$) revealed that the IOR of the for^{s2} homozygotes is recessive and is associated with the *dg2* locus, one of two genes encoding PKG in flies. The olfactory trap assay was designed with the intention of providing flies with a challenge resembling the location of a food source [42]. Mutants with altered ability to locate a food resource/odour and mutants which differ in their olfactory acuity or odour detection would be identified in this assay. Hence, for^{R} and for^{s2} flies were tested in the T-maze assay to determine if the IOR of for^{s2} flies resulted from an enhanced ability to locate a resource or from a general affect on odour detection.

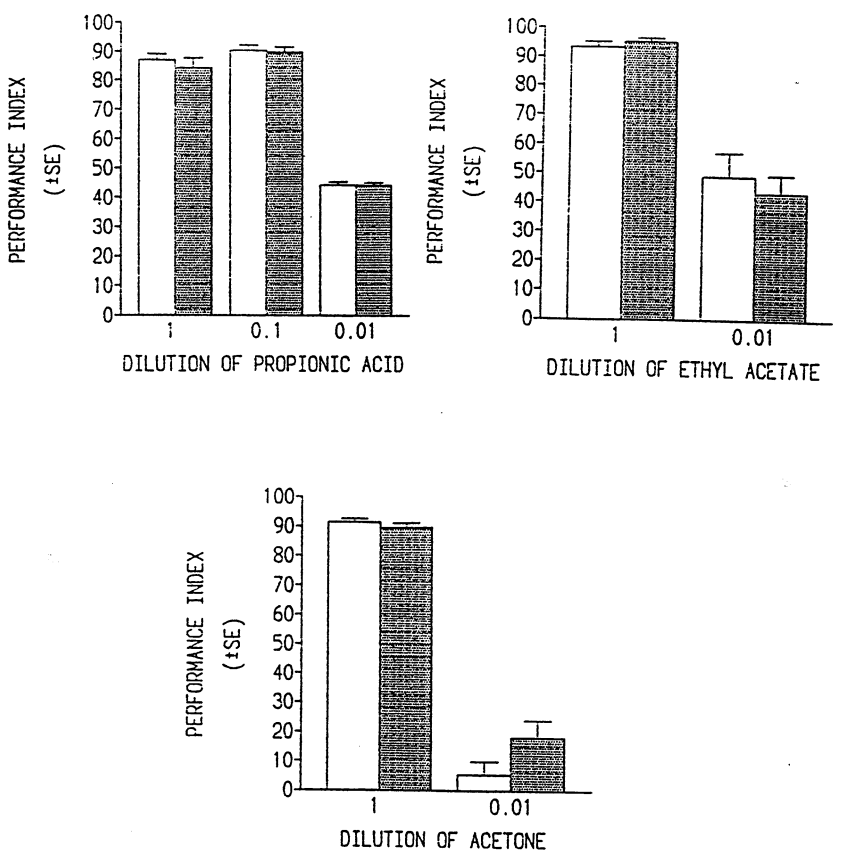

Fig. 3. Odour avoidance responses of for^{R} and for^{s2} flies to three odours. Performance indices (PIs) of adult groups of flies to pure ($7 \leq n \leq 8$), 10^{-1} ($n = 8$) and 10^{-2} ($n = 8$) dilutions (in mineral oil) of propionic acid; pure ($6 \leq n \leq 8$) and 10^{-2} ($6 \leq n \leq 7$) dilutions of ethyl acetate; pure ($n = 6$) and 10^{-2} ($n = 6$) dilution of acetone. One-way ANOVA detected no significant differences between the responses of for^{R} and for^{s2} flies for any of the odours. Bars represent mean PI $\pm$ S.E. Solid bars represent data for for^{R} flies while unshaded bars represent data for for^{s2}.

The performance index (PI) of for^{s2} and for^{R} flies did not differ with respect to the three odours tested in the T-maze assay suggesting that the for^{s2} mutation does not have a general affect upon odour detection. However, further analyses of this apparent olfactory anosmia including the examination of responses to a larger range of chemicals and concentrations are required before rigourous conclusions can be drawn regarding the odour specificity of the for^{s2} response. Odour specific anosmias are not unusual. In fact, a number of olfactory mutants cause defects in response to some odourants, but not others. Such odourant specificity is a phenotype expected of mutations which influence odourant specific transduction or processing pathways [42].

In addition to influencing adult olfactory trap response, the for^{s2} mutation is known to play a role in adult and larval foraging behaviour [32]. Thus, adult olfactory and foraging transduction pathways must use an overlapping set of genes. Such pleiotropy in *Drosophila* sensory systems is not unfounded [33,34,41,43]. The observation that the for^{s2} mutation does not influence the larval olfactory response to a food odourant suggests that lesions in the *for* gene result in adult specific olfactory defects. The *Drosophila* larval and adult olfactory organs differ in both morphology and developmental origin with the larval antenno–maxillary complex histolyzing during pupal metamorphosis and the adult antenna developing from an imaginal disc [15,26]. In addition, the re-organization of both the mushroom bodies (which are thought to be involved in the processing of peripheral sensory inputs) [16,39], and the formation of the antennal glomeruli (which are activated by chemical odours) [35], occur during pupal development. With the morphological and developmental dissimilarities of the larval and adult sensory systems, it may be that the role/sensitivity of the olfactory signal transduction pathways, and hence the role of PKG, also differ during these two life history stages. Alternatively, the role of PKG may have anatomical foci which influence both developmental stages (e.g. the developing central complex) [45].

Exposure to high doses of odourants has been shown to lead to a delayed, sustained elevation of cGMP concentration in olfactory receptor neurons, isolated rat olfactory cilia and insect antennae [9,12,44]. These elevated cGMP levels may play a role in adjusting the sensitivity of olfactory receptor neurons [11]. It is thought that this olfactory adaptation (reduction in response to an odour) is associated with modifications within components of signal transduction pathways [9,11]. For example, in insect antennae pretreated with cGMP there is a decrease in the second messenger response to odourant stimuli [11]. It is proposed that the higher levels of cGMP which are stimulated by exposure to an odourant could attenuate the responsiveness of receptor cells via proteins such as cGMP-activated protein kinases [11]. The results of this study provide evidence in support of this hypothesis.

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