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Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.)

Axel Decourtye,[a,*] Catherine Armengaud,[b] Michel Renou,[c] James Devillers,[d] Sophie Cluzeau,[e] Monique Gauthier,[b] and Minh-Hà Pham-Delègue[f]

[a] *ACTA, 18 avenue des Monts d'Or, 69890 La Tour de Salvagny, France*
[b] *Centre de Recherches sur la Cognition Animale, Université Paul Sabatier, 118 route de Narbonne, F-31062 Toulouse, France*
[c] *Unité de Phytopharmacie et Médiateurs Chimiques, INRA, Route de St. Cyr, F-78026 Versailles cedex, France*
[d] *CTIS, 3 chemin de la Gravière, F-69140 Rillieux La Pape, France*
[e] *ACTA, 149 rue de Bercy, F-75595 Paris cedex 12, France*
[f] *Laboratoire de Neurobiologie Comparée des Invertébrés, INRA, BP 23, La Guyonnerie, F-91440 Bures-sur-Yvette, France*

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Abstract

Imidacloprid is a chloronicotinyl insecticide which interacts with insect nicotinic acetylcholine receptors. Thirty minutes after oral treatment of honeybees with imidacloprid, the olfactory learning performances in a proboscis extension reflex (PER) procedure were impaired. In parallel, an increase of the cytochrome oxidase labelling was found into the calyces of the mushroom bodies. Imidacloprid administered 15 min or 1 h after a one-trial conditioning of PER impaired the medium-term olfactory memory. By contrast, the short-term (30 s or 3 min conditioning-treatment time interval) and long-term (24 h conditioning-treatment time interval) memories were unaffected. The impairment of medium-term olfactory memory by imidacloprid is discussed in the context of neural circuits suspected to mediate memory formation in the honeybee brain.

Keywords: Honeybee; Imidacloprid; Cytochrome oxidase; Olfactory learning; Proboscis extension reflex; Memory phases

1. Introduction

Imidacloprid is a chloronicotinyl insecticide effective against a wide range of arthropods, including aphids, scale insects, whiteflies, some heteroptera, coleoptera, and lepidoptera species [1]. Many studies have shown that imidacloprid had a high agonistic affinity with nicotinic acetylcholine receptors (nAChR)[1] of insects [2]. Nauen et al. [3] reported the binding site for imidacloprid on nAChR of honeybee head membrane

* Corresponding author. Fax: +33-4-78-19-62-29.
E-mail address: axel.decourtye@acta.asso.fr (A. Decourtye).

[1] *Abbreviations:* ALs, antennal lobes; CO, cytochrome oxidase; CS, conditioned stimulus; LD_{20}, lethal dose 20%; MBs, mushroom bodies; nAChR, nicotinic acetylcholine recepter; PER, proboscis extension reflex; US, unconditioned stimulus.

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preparations, as well as on cell bodies of the antennal lobes (ALs). Immunoreactivity against insect nAChR has been found in many regions of the honeybee brain, including areas involved in learning and memory processes, such as the ALs, the α-lobes, the calyces of the mushroom bodies (MBs) [4].

In the honeybee, learning abilities can be assessed using the conditioning of proboscis extension reflex (PER). The classical olfactory conditioning of the PER is based on the temporal paired association of an odorant stimulus (conditioned stimulus, CS) and a sucrose stimulation of the antennae (unconditioned stimulus, US). The PER is elicited by the antennal sucrose stimulation and is immediately rewarded by the uptake of same sucrose solution constituting a food reward. Following the training phase, a conditioned bee will exhibit the PER as a conditioned response to the CS alone [5,6]. A single pairing of CS/US is sufficient to condition honeybees and leads to short-term memory. Multiple learning trials lead to a high, stable and long-lasting memory (>4 days). The memory trace dynamics follow a model which assumes three kinds of sequential memories [7]. The short-term memory is lasting up to several minutes and is dominated by a non-associative and sensitisation component [8]. Consolidation of the associative component needs minutes to develop, and during this process memory becomes more specific to the CS. The consolidation processes lead to medium-term memory (several hours) and to long-term memory (several days) [7,9].

The acquisition process results in the capacity of the bee to establish CS/US association. The retrieval process leads to restore the conditioned response. Acquisition and memory retrieval processes taking place in the olfactory conditioning of the PER are impaired after injection into the calyces and α-lobes of MBs of mecamylamine, a nAChR antagonist [10]. Armengaud et al. [11] showed that the decrease of metabolism in the α-lobe following mecamylamine injection could be related to the memory impairment. Although the role of nAChR in the olfactory learning has been studied by analysing the effects of nAChR antagonists [10,12], the effect of nAChR agonists, such as imidacloprid, has not been considered. Therefore, in the present work, we aim to investigate more precisely the effects of imidacloprid on different parameters of memory during an olfactory conditioning of the PER. We have studied the sublethal effects of an oral treatment with imidacloprid on the acquisition and retrieval processes, as well as on the short- and medium-term olfactory memory. Complementary experiments were conducted to confirm the pharmacological action of imidacloprid on brain structures involved in memory processes (ALs, calyces, and α-lobes of the MBs), after oral administration of imidacloprid. In this purpose, the metabolic activity in the honeybee brain was investigated using cytochrome oxidase (CO) histochemistry. The principle of CO labelling is based on the metabolic activity of neurons. Changes in neuronal activity induce an increase of cell respiratory activity and an increase of enzymatic activity of the mitochondrial enzymes. The CO is the terminal enzyme in the electron transport chain of mitochondrial respiratory processes. The changes in CO activity in the central nervous system are concomitant to learning deficiencies [13]. Moreover, in invertebrates CO histochemistry is a valuable tool to identify brain structures involved in memory processes [14,15]. In a previous work, Armengaud et al. [16] showed that CO histochemistry could be used to identify the target structures of cholinergic ligands in the honeybee brain and particularly those of imidacloprid.

2. Materials and methods

2.1. Chemicals

Imidacloprid (98% purity, Cluzeau Info Labo, Sainte-Foy-La-Grande, France) was dissolved in acetone, and diluted in sucrose solution ($300\,g\,L^{-1}$) to obtain the following final concentrations of the product: $25\,mg\,L^{-1}$ and $250\,\mu g\,L^{-1}$. The final concentration of acetone in sucrose solution was of 1% (v/v). For the treatment, bees were fed 0.5 μl of the appropriate dilution of imidacloprid in sucrose (2-μl Gilson micropipette). Consequently, imidacloprid was tested at doses of 12 and 0.12 ng per bee. The highest dose tested corresponds to the lethal dose 20% (LD_{20}), i.e., the dose at which 20% of the

individuals died 24 h after treatment (Decourtye, unpublished data). Control group received 0.5 μl of sucrose solution (300 g L^{-1}, 1% acetone v/v).

2.2. Histochemistry study

2.2.1. Insects

Worker honeybees (*Apis mellifera*) were kept in a cage [17] with water and food (honey) ad libitum and placed in an incubator (25 ± 2 °C, 55 ± 10% RH, darkness) overnight. The experiment was repeated on six honeybees for each of the three treatment modalities (12 and 0.12 ng per bee of imidacloprid, and control).

2.2.2. Cytochrome oxidase staining

The day after the collection of bees, the histochemistry of cytochrome oxidase was carried out according to the technique of Armengaud et al. [16]. Thirty minutes after the oral insecticide treatment, the worker bees were anaesthetised using CO_2 (15 s, 4×10^4 Pa pressure) and killed by decapitation. This time interval between the imidacloprid treatment and the sacrifice was chosen because it allows the presence of the parent compound and its metabolites into the brain after oral administration [18]. After treatment, brains were dissected in a fixative solution (4% paraformaldehyde in phosphate buffer 0.1 M). Cryostat frontal sections (16 μm) of the whole brain were prepared and were incubated for a period of 30 min. The incubation medium consisted in 0.02% cytochrome-*c*, 0.06% diaminobenzidine, 4.5% sucrose in phosphate buffer 0.1 M. Densitometry analysis of the sections was conducted under 20× magnification (Zeiss microscope). Quantification was carried out by computer-aided densitometry of CO histochemistry staining intensity using Photoshop (version 6.0, Adobe) image analysis software. Densitometry analysis was performed for ALs (cortical and medullar neuropiles), MBs (lip and basal ring of calyces), and α-lobes (B1, B2, and B3). It was decided by Armengaud et al. [16] to call the main layers of α-lobe revealed by CO histochemistry: B1, B2, and B3. These bands represent functionally distinct zones rather anatomic distinct zones such as those revealed by Golgi and immunocytological studies [19,20].

2.2.3. Data analysis

The mean of grey level of three to six sections of each analysed structure was calculated for control and treated groups. For lips and basal ring of the MBs, the grey levels of median and lateral structures measured for each section were pooled for data analysis. The grey levels of the different brain structures were compared among treatments using a one way ANOVA ($P < 0.05$). When the F value was significant, the Fisher's least significant difference (LSD) test was used to grade the different treatment groups ($P < 0.05$).

2.3. Olfactory learning study

2.3.1. Insects

Emerging workers bees were collected from brood combs of outdoor hives. They were caged in groups of 50–60 individuals [17], maintained in an incubator at 33 ± 2 °C, 40 ± 10% RH, and fed candy sugar, and water ad libitum during the overall rearing period, and pollen during the first eight days. Fourteen- to sixteen-day-old bees were used in the experiments since they give the most consistent performances in the olfactory conditioning of the PER [21]. Bees were individually mounted in glass holders, leaving their antennae and mouthparts free. They were starved for 4–5 h prior to conditioning.

2.3.2. Reflex response

The effects of imidacloprid on the responsiveness to sucrose and odour stimuli were tested 30 min after the oral treatment. On healthy individuals, application of sucrose solution (300 g L^{-1}) to the antennae uses to elicit PER (reflex response). Factors that disrupt this response potentially affect the sensory-motor components of the PER.

2.3.3. Conditioning trials

The classical olfactory conditioning of the PER was carried out as previously described by Bitterman et al. [5] and Sandoz et al. [6]. Only the bees that showed a PER after sucrose stimulation to the antennae were used for the experiments. A sucrose solution (300 g L^{-1}) was used as the unconditioned stimulus (US). An odour pulse (linalool, 95–97%

purity, Sigma) delivered after a 15 s exposure to the airflow was used as the conditioned stimulus (CS). During each conditioning trial, the CS was delivered for 6 s. After the first 3 s, the sucrose solution was presented to the antennae, resulting in a PER. Then the reward (food uptake) was provided to the bees with the same sucrose solution.

2.3.4. Test trial

In the testing trial, the CS was presented alone. Along this procedure, we evaluated whether the bee responded to the odour alone.

2.3.5. Experiment 1. Pre-training treatment

To analyse the effects on the acquisition process, oral treatment with imidacloprid was administered 30 min before three successive conditioning trials (C1–C3). This delay was chosen according to the protocol of the histochemistry study. Delay between trials was of 20–30 min (Fig. 1A). Daily experiments comprising bees subjected to imidacloprid (0.12 or 12 ng per bee), and untreated control bees were repeated until the samples of tested bees reached ca. 30 individuals per treatment.

2.3.6. Experiment 2. Concomitant-training treatment

To assess the effects on retrieval performances, imidacloprid was administered in the reward (0.5 µl sucrose solution) during a one-trial conditioning. The conditioned response was tested 30 s, 3 min, 15 min, 1 or 24 h after the conditioning trial (Fig. 1B). The time intervals between the conditioning trial and the retention test (CS presentation) were chosen in order to take into account the temporal dynamics of honeybee memory [7]. When the test was performed 24 h after the conditioning trial, the bees were fed with a sucrose solution ($500\ g\ L^{-1}$) until satiation after the conditioning session. Each of the two doses of imidacloprid (12 and 0.12 ng per bee) was tested independently with a control group.

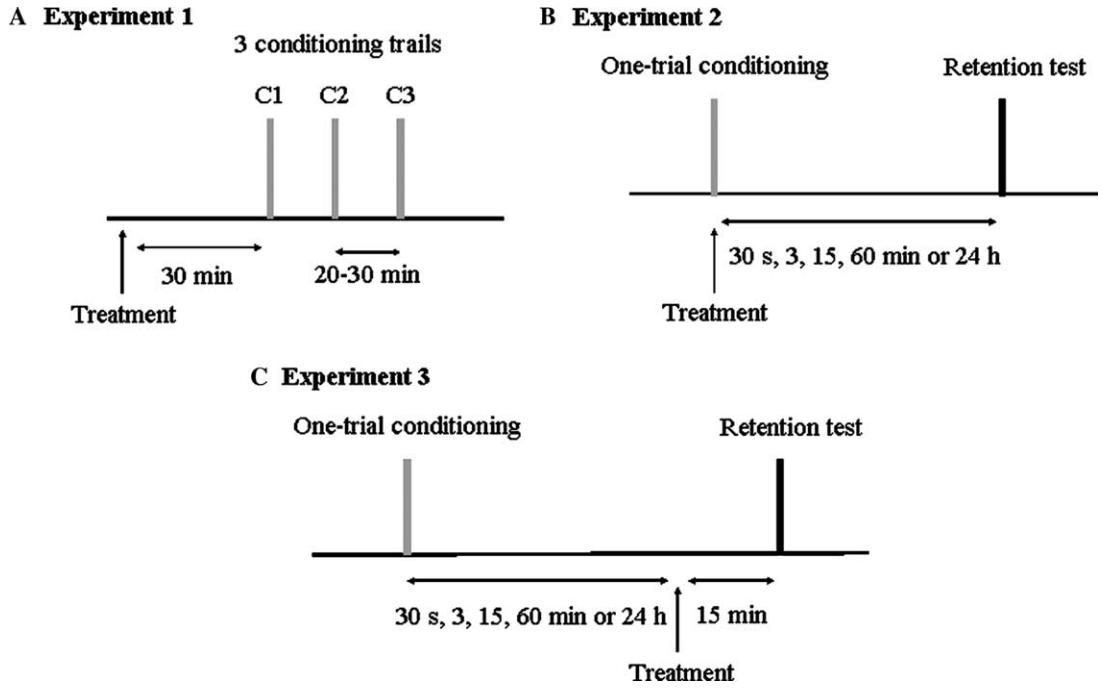

Fig. 1. Scheme of the behavioural paradigm used to test imidacloprid effects on acquisition (A, Experiment 1), retrieval (B, Experiment 2), and short-, medium-, and long-term retention (C, Experiment 3) of olfactory memory.

2.3.7. Experiment 3. Post-training treatment

The effect on short-, medium-, and long-term retention was studied with the oral treatment of imidacloprid after one-trial conditioning. The treatment was carried out 30 s, 3 min, 15 min, 1 or 24 h after conditioning (Fig. 1C). The retention performances were evaluated 15 min after treatment (as no effect on retrieval was observed at this delay; see Experiment 2). Bees not responding to odour stimulus were tested for reflex response by directly touching the antennae with sucrose solution (300 g L^{-1}) to ensure that the motor component of the reflex was intact. Only the dose of 12 ng per bee was used to test the effects of imidacloprid on short-, medium-, and long-term retention. Every testing day was organised as follows: bees exposed to imidacloprid and untreated control bees were tested, leading to a total of 40–50 bees tested per day, with 3–11 bees for each group. This was done repeatedly, until about 40–50 bees per group were obtained. For the study of retrieval performances and memory phases (Experiments 2 and 3), bees were tested with odour alone only once.

2.3.8. Data analysis

In Experiment 1, the conditioning rate was compared among the three treatment groups using a χ^2 test in a contingency table procedure (2 df, $P < 0.05$). When the distribution was found to be non-uniform, two-by-two comparisons of the number of responses between the treated and the control bees were carried out using a χ^2 test with 1 df. The significance threshold was corrected according to Dunn-Sidak method [22]. The significance level was $\alpha' = 1 - (1 - \alpha)^{1/k}$, where k was the number of intended tests ($\alpha' = 0.025$). In Experiments 2 and 3, the number of responses between the treated and the control bees were carried out using a χ^2 test with 1 df ($P < 0.05$).

3. Results

3.1. Histochemistry study

Thirty minutes after oral treatment with imidacloprid, a significant increase of CO staining was observed in lip ($F = 19.2$, 3 df, $P < 0.001$) and basal ring ($F = 6.4$, 3 df, $P < 0.001$) of the MB calyces (Table 1). In the lip, the two doses of imidacloprid (12 and 0.12 ng per bee) induced a significant increase of the CO labelling. Differently, only the highest dose of imidacloprid increased the labelling in the basal ring. No significant treatment effect was noted in the α-lobe and ALs.

3.2. Olfactory learning study

3.2.1. Mortality

No difference was found in the mortality rate of the different groups (0.12 ng per bee: 11%; 12 ng per bee: 13%; control: 12%; $\chi^2 = 1.5$, 2 df, $P > 0.05$; $N = 250$–300). Although the highest

Table 1
Effect of imidacloprid on CO histochemistry in calyces of the mushroom bodies (MBs), α-lobe, and antennal lobes (ALs)

Brain structures		Treatments		
		12 ng	0.12 ng	Control
MBs	Lip	78.2 ± 1.6 (a)[a]	80.6 ± 1.0 (a)	74.3 ± 1.0 (b)
	Basal ring	100.0 ± 1.6 (a)	98.5 ± 0.8 (b)	95.1 ± 0.8 (b)
α-Lobe	B1	67.8 ± 5.1	62.5 ± 4.4	61.4 ± 3.9
	B2	59.0 ± 3.2	55.3 ± 3.2	52.1 ± 3.1
	B3	97.1 ± 6.6	92.1 ± 4.6	91.5 ± 5.1
ALs	Cortical area	103.7 ± 2.5	114.0 ± 3.5	104.3 ± 3.6
	Medullar area	75.5 ± 2.4	87.1 ± 4.9	77.6 ± 3.1

Note. Mean ± SEM of optical density, expressed as grey level; $N = 6$.
[a] Different letters indicate different staining level with $P < 0.05$.

dose was initially estimated as inducing 20% of dead bees (LD_{20}), the two treatments with imidacloprid were sub-lethal.

3.2.2. Reflex response

At least 83% of bees showed a clear PER following antennal sugar stimulation (Table 2). No effect of imidacloprid was found on the responsiveness of the bees to the sucrose solution alone, whether the test was carried out 30 min after treatment or along the different trials of the conditioning procedure (60–90 min after treatment) (χ^2, 2 df, $P > 0.05$, in all cases; Table 2).

3.2.3. Experiment 1. Pre-training treatment

Fig. 2 shows the percentage of bees responding to CS (conditioned responses) for each learning trial (C1–C3) in groups fed of imidacloprid (0.12 or 12 ng per bee) and in the control bees fed of sucrose. Thirty minutes after treatment with imidacloprid at the highest dose (12 ng per bee), bees showed response level lower than that of untreated bees: 25 and 31% conditioned responses at C2 and C3, respectively, versus 88 and 92% in the control ($N = 26$–32). Bees treated with this dose exhibited significantly lower performances compared to the response of the control group (C2: $\chi^2 = 23.2$; C3: $\chi^2 = 22.0$; 1 df, $P < 0.001$ for both). No significant difference was found between treated bees at lower dose (0.12 ng per bee) compared to the control bees.

3.2.4. Experiment 2. Concomitant-training treatment

When CS was presented at different times after a one-trial conditioning with an imidacloprid-added reward, we observed a significant decrease in the groups treated at a dose of 12 ng per bee and tested 1 or 24 h after imidacloprid treatment (1 h: $\chi^2 = 22.2$; 24 h: $\chi^2 = 27.3$; 1 df, $P < 0.001$ for both; Fig. 3). In the control group, 93 and 84% of bees

Table 2
Effects of imidacloprid on reflex responses

Treatment	Pre-conditioning	Conditioning trials		
		C1	C2	C3
12 ng	83.7	87.5	81.2	68.7
0.12 ng	92.0	92.3	96.1	92.3
Control	92.5	92.3	96.1	96.1

Note. Percentage of PER to sucrose solution applied to the antennae; pre-conditioning: $N = 50$; conditioning trials: $N = 23$–32.

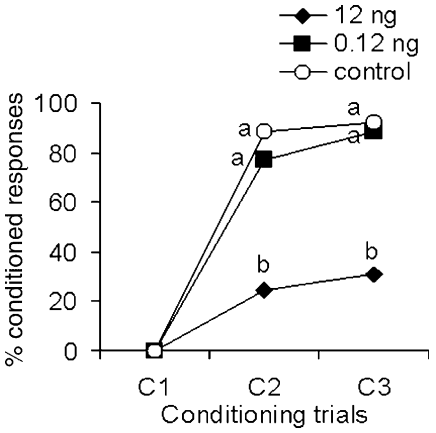

Fig. 2. Effects of imidacloprid on acquisition process (Experiment 1). $N = 23$–32. Comparisons of the number of conditioned responses among groups at each trial were done using χ^2 test (2 df, $P < 0.05$, NS, non-significant). When significant, it was followed by two-by-two comparisons (χ^2 test, 1 df, $P < 0.025$). Different letters indicate significantly different response levels.

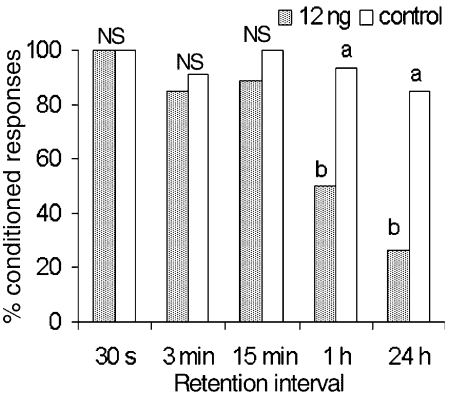

Fig. 3. Effects of imidacloprid on retrieval of olfactory memory (Experiment 2). During a one-trial conditioning, bees were rewarded with 0.5 μl of either imidacloprid-added or control sucrose solution. The retention was tested 30 s, 3 min, 15 min, 1 or 24 h after the conditioning. $N = 22$–49. The numbers of conditioned responses obtained during the retention trials were compared among treatment using χ^2 test (1 df, $P < 0.05$, NS, non-significant). Different letters indicate different response levels.

showed a conditioned response at 1 and 24 h, respectively $N = 39$–46. For these same delays, lower levels of responses were obtained with the highest dose of imidacloprid, reaching 50 and 26% of conditioned responses, respectively $N = 42$–48. At lower dose (0.12 ng per bee), imidacloprid induced no effect compared to control rate whatever the retention interval (30 s: $\chi^2 = 0.6$; 3 min: $\chi^2 = 0.3$; 15 min: $\chi^2 = 0.01$; 1 h: $\chi^2 = 0.2$; 24 h: $\chi^2 = 0.00$; 1 df, $P > 0.05$, in all cases; data not shown). For all delays of retention, 71–91% of conditioned responses was observed in treated bees with this dose ($N = 30$–35) and 77–97% was observed in untreated bees ($N = 33$–40).

3.2.5. Experiment 3. Post-training treatment

The post-training treatment at 15 min and 1 h led to significant differences in medium-term retention between treated bees (12 ng per bee) and control bees (15 min: $\chi^2 = 9.7$; 1 h: $\chi^2 = 6.0$; 1 df, $P < 0.05$ for both; Fig. 4). Of the treated bees tested ($N = 31$–38), 82 and 92% showed a conditioned response for the delays of 15 min and 1 h, respectively, versus 92 and 100% in control group ($N = 44$–46). When imidacloprid was applied 30 s, 3 min or 24 h after conditioning, the conditioning rate was comprised between 64 and 83%, with no difference between treated bees and control group (30 s: $\chi^2 = 0.7$; 3 min: $\chi^2 = 1.6$; 24 h: $\chi^2 = 1.5$; 1 df, $P > 0.05$, in all cases; $N = 31$–43). Thus, imidacloprid applied after conditioning impaired medium-term retention but not short- and long-term retention.

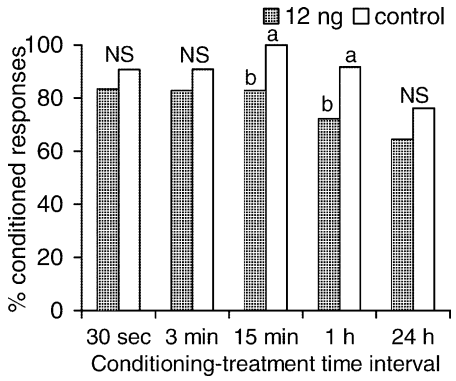

Fig. 4. Effects of imidacloprid on the retention of short- and medium-term memory (Experiment 3). At different times after a one-trial conditioning, bees were fed with 0.5 µl of either imidacloprid-added or control sucrose solution. The retention was tested 15 min after the treatment. $N = 40$–50. The numbers of conditioned responses obtained during the retention trials were compared among treatment using χ^2 test (1 df, $P < 0.05$, NS, non-significant). Different letters indicate different response levels.

4. Discussion

Since the discovery that nAChR are localised in many regions of the honeybee brain [4,23], they have been implicated in the modulation of learning and memory processes [10,12]. Here, we presented a behavioural and histochemical analysis of the effect of imidacloprid, a nAChR agonist, on the olfactory learning in the honeybee. The behavioural results indicated that imidacloprid at a sublethal dose (12 ng per animal) decreased the acquisition and the retention performances tested in the conditioned PER paradigm. Furthermore, this treatment induced a negative effect on the medium-term retention (15 min or 1 h conditioning-treatment time interval), but not on the short-term retention (30 s or 3 min conditioning-treatment time interval). Our results might be interpreted as an action of imidacloprid on retrieval or on memory formation. If an action on retrieval has occurred, one would predict that retention should always be impaired when tested 15 min after treatment. Indeed, the retention was tested 15 min after treatment in all cases of conditioning-treatment time intervals. However, treatment 30 s, 3 min or 24 h after conditioning leaves retention intact. Therefore, the impairment of medium-term retention is likely due to the impairment of memory formation rather than of retrieval. We assume that the consolidation process which ensures the transfer from short-term memory to medium-term memory (highly specific) within 10–15 min after the conditioning trial [7,8] was affected by imidacloprid. Thus, the long-term memory lasting 1–2 days after conditioning, called "early long-term memory" [7,9], was not impaired by imidacloprid-treatment applied after the consolidation phase (conditioning-treatment time interval of 24 h).

The time-dependent effect of imidacloprid might also be related to the metabolisation of

imidacloprid. Distribution kinetics study of radiolabelled imidacloprid showed that metabolites products are detected in honeybee heads only 20 min after oral administration [18]. Now, some imidacloprid metabolites, such as olefin, 5-hydroxy-imidacloprid and 4/5-hydroxy-imidacloprid, may also be highly toxic to honeybees [3,24]. Therefore, disruption of retention tested 1 or 24 h after imidacloprid treatment might account for the effects of imidacloprid metabolites, rather than a direct effect of the test compound. On the other hand, the effect on the medium-term memory should be mainly due to imidacloprid since it was found only 15 min after treatment.

The question arises of which mechanisms underlie the impairment of medium-term retention by imidacloprid. Two points are in favour of an effect of imidacloprid on brain structures involved in the memory formation, as the critical factor. First, imidacloprid affects the central nervous system of the honeybee. Using CO histochemistry to carry out metabolic mapping of discrete brain regions, we showed that the oxidative metabolism in the calyces of the MBs is increased 30 min after oral treatment with imidacloprid. The histochemistry of CO is used as an endogenous metabolic marker for neuronal activity: neuronal activity demands energy and consequently increases oxidative energy production [25]. The increase of oxidative metabolism in the brain after 12 ng imidacloprid oral treatment is consistent with results obtained with nicotine and imidacloprid intracranial injection to the same dose [16]. Additionally, the hypothesis of a disruption of motor neural circuit of the PER cannot explain our results since imidacloprid has no effect on the reflex response. Moreover imidacloprid did not affect the sensitivity to linalool odour as indicated by electroantennogram recordings (Decourtye, unpublished data). Thus, the performances decrease noticed for medium-term memory cannot be ascribed to imidacloprid-induced modifications of motor activity or peripheral olfactory sensitivity. Second, it is generally assumed that memory formation occurs at multiple sites in parallel, including the ALs and the MBs [26]. Although there is no evidence whether particular memory components are stored preferentially in any of these neuropiles, Menzel [7] assumes that the medium-term memory trace in the ALs (increase of protease-dependent PKC activity) is established under the guidance of the MBs. As indicates CO histochemistry treatments affect the neural activity in the MBs, but not in the ALs, our results indicate that only memory-related neural plasticity localised in the MBs would be affected by imidacloprid.

Several studies support the fact that imidacloprid acts at the level of the MB calyces. The Kenyon cells, which are intrinsic neurones of the MB calyces, express the main target of imidacloprid, the nAChR [27–29]. Thus, the metabolism changes that we observed in the MB calyces could rely on the stimulation of Kenyon cells by imidacloprid. On an other hand, electrophysiological studies in cockroach have provided evidence that imidacloprid can depolarise the giant interneurones and increase the spontaneous activity of the corresponding synaptic pathway, these effects being followed by a blocking of the cholinergic synaptic transmission [30]. In this previous work, the high neuronal activity should be followed by a demand of energy, an increase of oxidative energy production, and consequently an increase of CO activity. Therefore, the increase of CO activity observed in our study reveals without any doubt an increase of neuronal activity in the calyces of the MBs, and a possible subsequent blocking of cholinergic synaptic transmission. This suggests that imidacloprid action on the Kenyon cells of the honeybee can lead to a blocking of neuronal transmission, and consequently to a disruption in Kenyon cells contribution to medium-term memory formation.

An inhibitory effect of imidacloprid on a learning task was observed in the present work at the highest dose (12 ng), whereas this insecticide had been reported to facilitate another type of learning, the PER habituation with a 10-fold weaker dose [31]. The habituation is characterised by a decline of PER to a repeated antennal sucrose stimulation. In 7-day-old bees, treatment with imidacloprid leads to an increase in the number of trials necessary to abolish the response, whereas in 8-day-old bees, it leads to a reduction in the number of trials for habituation [32]. Comparing our results with those previously published, we can

hypothesise that imidacloprid has variable behavioural effects in the honeybee according to the dose and the learning tasks (associative paradigm such as olfactory conditioning of PER, or non-associative paradigm such as habituation of PER). Moreover, we assume that the different behavioural effects of imidacloprid vary according to brain structures underlying the memory processes. Indeed, the habituation implies the formation of a non-associative memory, which would be mainly localised in the ALs [33], whereas the olfactory associative conditioning of the PER induces the formation of an associative and contextual memory, for which the MBs are the critical substrate [34].

Finally, treatment with imidacloprid resulted in a specific effect on the medium-term retention. These results showed that consolidation is altered in imidacloprid-treated bees. The structure-specific increase of CO activity into the MBs observed after treatment suggests that imidacloprid impairs olfactory memory by a physiological effect at the MBs level. The precise reasons for the amnesiac effect of imidacloprid are still unclear; imidacloprid may affect the Kenyon cells contribution to olfactory memory formation.

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