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OBJECT INDIVIDUATION IN DOMESTIC CHICKS (*Gallus gallus*)

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ABSTRACT

Object individuation is the process by which organisms establish the number of distinct objects present in an event. The ability of individuating objects was investigated in two/three-day-old chicks (*Gallus gallus*). A first series of experiments (Exp. 1 - Exp. 6) assessed the role of the property information provided by colour, shape, size or individually distinctive features, as well as spatiotemporal information in object individuation. A second series (Exp. 7 - Exp. 10) aimed at investigating the ability to use property/kind information using imprinting objects and food items (i.e. mealworms) as stimuli of different category. Newborn chicks were exposed (i.e., imprinted) to sets of objects which were different or identical for property and property/kind information, and the chicks' spontaneous tendency to approach the larger group of imprinting objects and food items was exploited. Each chick underwent a free choice test in which two groups of events were shown: a group comprised two different stimuli (i.e. for property or for kind); the second group was composed by a single stimulus presented twice. Every stimulus in each group of events was sequentially presented and concealed in the same spatial location and the number of events taking place at each location was equalized (Sequential Presentation test). Chicks spontaneously approached the two different objects rather than the single object seen twice. A possible preference for the more varied set of stimuli was excluded by testing chicks in a simultaneous presentation of two different objects *Vs.* two identical objects (Simultaneous Presentation test). Moreover, use of spatiotemporal information was assessed through simultaneous presentation of three identical objects *Vs.* two different objects. When increasing the number of presentations of the single stimulus (up to 3 times) and comparing it with two different stimuli presented once each, chicks correctly individuated the larger group of imprinting objects only if objects were all different from one another (i.e. distinctive features had been put on each object). Any role of experience was excluded by presenting chicks with stimuli of a completely novel colour with respect to the original colour of the imprinting stimuli. Results show that chicks are able to use the property information provided by colour, shape, size or individually distinctive features, spatiotemporal information and property/kind information provided by social and food categories for object individuation. The fact that object individuation is precociously available in the young of a vertebrate species suggests it may depend on inborn biological predispositions rather than on experiential or language-related processes.

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INTRODUCTION

In everyday life, humans organize their visual world by automatically parsing external input into separate objects, forming representations of such objects and tracing their identity through time and space.

There are two fundamental capacities for object representation: one is the object segregation that consists in separating visual arrays into individual objects, and the other is the object individuation that consists in keeping track of these objects through time and space. In other words, the former process assigns surfaces to distinct objects, the latter assigns segregated objects seen on different occasions to single or multiple objects (for a review see Xu, 2007).

My doctoral thesis is focused on the investigation of the process of object individuation from a comparative perspective, employing the chick of domestic fowl (*Gallus gallus*) as an animal model.

Core knowledge

The aim of comparing data from different species is to comprehend the phylogenesis of the cognitive abilities investigated (Platt & Spelke, 2009). The underlying assumption being that cognitive processes are firstly adaptive specializations developed to solve specific problems in the natural everyday environment. According to this view, comparative researches on cognition are carried out in order to shed light on the understanding of conceptual knowledge in humans. A prominent theory about the origin of human knowledge argues that human beings are endowed with a set of core systems for representing significant aspects of the environment which is called “core knowledge” (Platt & Spelke, 2009) or “core cognition” (Carey, 2009). These core knowledge systems form the building blocks for uniquely human skills. The complexity of human cultural-specific skills is drawn on this set of core systems that are psychological and neural mechanisms with two general distinct properties: they evolved before humanity and thus are shared with other animals, and they emerged early in human development and are thus common to infants, children and adults (Hauser & Spelke, 2004). In other words, these cognitive mechanisms are shared with non-human animals, are present at birth and operate throughout life as domain-specific learning devices.

Core cognition comprises mental structures that represent core domains (or systems): those core domains are highly innate structured mechanisms designed by the natural selection for representing relevant classes of entities in the world. Such mechanisms create a causal connection between real-world entities and representations in core cognition.

Like sensory and perceptual features of the world, entities in core domains of knowledge are identified by modular innate perceptual-input devices. However, representations in core cognition differ from sensory and perceptual representations in having a rich, conceptual and inferential role in thought (Carey, 2009). Moreover, representations from distinct core cognition systems are inferentially integrated (i.e. these conceptual processes are central), while sensory and perceptual representations are independent (i.e. these processes are encapsulated; Fodor, 1983).

Core cognition differs also from the explicit conceptual representations that articulate intuitive theories: the conceptual role of the concepts in core cognition is less rich than that of the concepts embedded in intuitive theories.

Systems in core cognition have several specific properties. They are domain-specific: each system functions to represent particular kinds of entities. They are task-specific: each system functions to solve limited set problems. They are relatively encapsulated: each system operates with a fair degree of independence from other cognitive systems. Each system is relatively automatic and impermeable to explicitly held beliefs and goals.

What is relevant for the present research is that comparative and developmental studies support the idea that human cognition is founded on five systems for representing objects, actions, numbers, space and social partners. Each system has deep roots in human phylogeny and ontogeny (for a review see Spelke & Kinzler, 2007).

Considering the core system of object representation, it has been revealed that human infants, as well as other animals, perceive object boundaries in order to represent the complete shapes of objects moving partly or fully out of view (Aguiar & Baillargeon, 1999; Spelke, 1990). Some of these abilities are observed in absence of any visual experience in newborn human infants (Valenza, Leo, Gava, & Simion, 2006) and newly hatched chicks (Regolin & Vallortigara, 1995). Similarly, non-human primates showed to represent objects in continuity and contact constrains (e.g. Santos, 2004). Interestingly, the same system seems to govern human adults' process underlying object directed attention (see Scholl, 2001) and seems to be consistent across human development, as shown by the behaviour of Pirahãs, a remote Amazonian Group which have been reported to differ dramatically in their language, culture and cognitive abilities from most of other contemporary human groups (Everett, 2005).

In particular, regarding the animal model employed in this research, it has been already demonstrated that chicks can represent partly occluded objects through amodal completion (Regolin & Vallortigara, 1995), and can spontaneously represent the possible version of a three-dimensional object (choosing the two-dimensional image that depicted the possible rather than the impossible version of that same object) after being exposed to a two-dimensional image in which junctions providing cues to global structure were occluded (Regolin, Rugani, Stancher, & Vallortigara, 2011).

Up to now, however, it is unclear whether chicks represent objects making use of information provided by the categories to which objects belong or by their different properties for object individuation.

The present research aims at addressing this issue, contributing to understand the foundations of core system for object representation.

Object individuation

In the cognitive literature, object individuation is defined as the process that allows to determine the number of objects in an event and to establish their numerical identity. Recognizing identity for an object carries with it the need to recognize individuality, since the question of identity concerns identity of an individual. Individuality in turn raises the question of how many objects one is dealing with, even if “exactly one” is the answer. The twin problem of object individuation and identity has come to dominate current work on the “object concept” (Leslie & Kaldy, 2001). More broadly, object individuation answers to the question: is it the same object that I have seen in two different times? Or are they two different objects? It allows to establish how many objects are present in an event (i.e. numerical identity).

A conceptual distinction has been drawn between object individuation and object identification within the object-indexing framework (Leslie, Xu, Tremoulet, & Scholl, 1998).

Object identification refers to the process of biding property information to the existing object representations such as that object can be re-identified at another time. It answers the question “which one?”.

Sortal concepts

In order to answer the question about ”how many?” or “is it the same?” we need to specify “how many (or is it the same of) what”. In the literature, the “what” is defined as a sortal concept, which is a concept that provides principles of individuation and principles of identity (Hirsch, 1982; Wiggings, 1980). Our identity criteria is sortal-specific (relative), in the sense that the same property difference may or may not indicate a change in identity depending on the kind of object considered. A change in size or colour or shape indicates a change in identity for a mug, but not necessarily for a plant. On the other hand, a person may not be “the same BABY” as it was 20 years ago, although she might still be the same PERSON (Xu, 2007; see also Xu, 1997 for a discussion about sortal concepts).

Sortals are defined as the concepts that provide the criteria to enumerate and track identity over time, and they are lexicalized as count nouns in languages that make the count-mass distinction (Baker, 2003; Hirsch, 1982; Wiggings, 1980). All concepts provide principle of application (i.e. specifying what falls under the concept), but not all the concepts provide principle of individuation and identity. For example, consider the concept RED. We cannot count THE RED unless we specify a sortal, for example from a general “red objects” to a more specific “red mugs”, “red socks” etc. Similarly, we cannot ask whether something is the same red unless we mean the same red of something. Mass nouns do not provide principle of enumeration and identity in the straightforward way (e.g. water or sand; see Xu, 2010).

Many research groups have been interested in investigating the development of the representation of sortal concepts. In particular, the goal is to study how concepts are individuated and acquired, how concepts might support individuation, categorization and inference, and how concepts underlie word meanings. The studies focused on infants in order to understand how sortal concepts develop in our species and on two non-human mammals species in order to understand their generality in a comparative perspective; these research branches are related to how learning of language, and in particular of count nouns, may play a role in constructing these concepts.

Information used in object individuation

Image this scenario: an object is seen at time 1 and then an object is seen at time 2. The question arises as to whether the same object is seen on two different occasions or whether two distinct objects are present. What human adults do is to individuate objects as distinct on the basis of, at least, three sources of information (Spelke, 1990).

1) *Spatiotemporal information* is information about object location and object motion. It includes generalization such as objects travel on spatiotemporal continuous paths, the same object cannot occupy two places at the same time and two objects cannot be at the same place at the same time. That means that the red mug left in the kitchen this morning could not be the same red mug found later, that same morning, on the desk of the office because the same object cannot be at the same time in two different places and it cannot travel from one place to another by itself. Adults

represent two distinct objects if there is no spatiotemporally continuous path that could unite them.

2) *Property information* includes generalizations such as objects usually do not change in their features, such as colour, size, shape or pattern. The red mug on the desk is not the same object as the green mug seen earlier on the same spatial position.
3) *Kind/sortal information* includes generalization such as objects to not usually change kind membership. It specifies categorization under concepts (e.g. duck, ball, cup, car etc.), categories of objects united by functional/causal features as well as by perceptual features. Adults draw on kind/sortal information in object individuation when they conclude that the red mug on the desk cannot be the same object as the red pencil seen earlier on the same desk or that the dog that went behind a tree cannot be the same individual as the cat we found in the same location in a different moment.

The definition of spatiotemporal information is valid for every object, independently from its category, whereas, property information is kind/sortal specific: the small plant bought for your office two months ago is the same bush that now overruns the office. The plant has changed in size (and it might have changed also in colour) but it is still the same plant. On the contrary, having a red mug on a desk and then finding a green mug on the same desk indicates that those are probably different objects.

Object individuation in infants

Infants have a fundamental sense of physical objects as discrete, cohesive, bounded volumes that exist in a continuous fashion in time and space (e.g. Spelke, 1990; Spelke, 1994; Spelke, Kestenbaum, Simons, & Wein, 1995).

One methodology used for studying object individuation employed the “violation-of-expectation” paradigm, which exploits infants’ tendency to look longer at event-outcomes if they are “surprising”: namely if they contradict or violate the infants’ understanding (and therefore expectancy) of the event. In the typical paradigm for the investigation of object individuation, infants are presented with two identical screens (see Fig. 1 from Xu & Carey, 1996). Then, one object pops out from behind one screen and returns behind it; thereafter, a physically identical object pops out from the other screen and then returns behind it. No objects appear in the space between the two screens. The assumption is the following: because objects travel on spatiotemporal continuous paths, the spatiotemporal discontinuity provides evidence that there are two, though identical, objects in the event, one behind each screen. In the test trials, the screens are removed and infants’ looking times for each outcome are recorded as dependent variable. Results demonstrated that, when presented with two featurally-identical objects in spatiotemporal discontinuity, infants preferentially looked at the unexpected outcome of one object than at the expected outcome of two objects demonstrating to expect two identical objects being involved in the event.

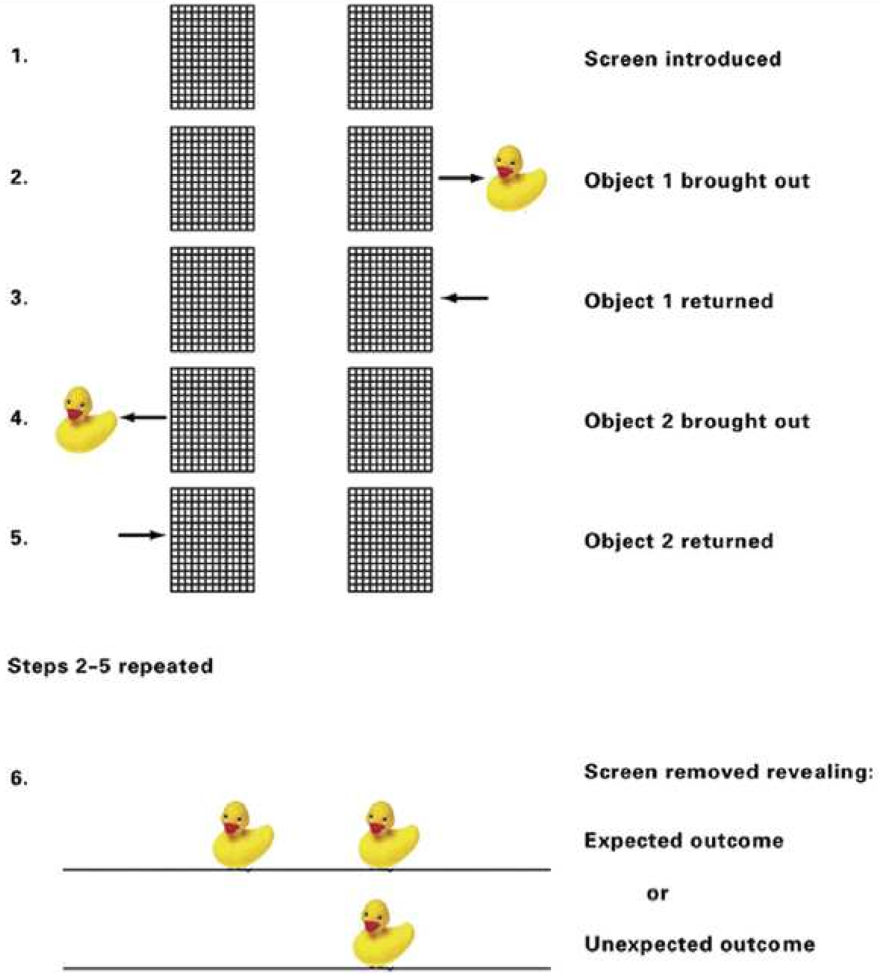


Fig. 1. Example of the procedure used for investigating spatiotemporal information (from Xu & Carey, 1996). Two featurally-identical objects are presented in spatiotemporal discontiunity. Infants' looking times for the expected outcome of two objects and the unexpected outcome of one object were recorded.

Infants demonstrated to precociously possess the knowledge that objects travel on spatiotemporal continuous paths and that the same object cannot be at the same time in two different places (at 2 months: Aguiar & Ballairgeon, 1999; at 4 months: Spelke et al., 1995; at 5 months: Wynn, 1992; at 10 months Xu & Carey, 1996).

But, what happens if featurally different objects are presented in spatiotemporal continuity? Answering to this question consists in the investigation of infant's ability to use property/kind information. This issue has been assessed by employing both preferential looking (Spelke, 1990; Xu & Carey, 1996; Xu, Carey, & Welch, 1999) and manual search (Van de Walle, Carey, & Prevor, 2000; Xu & Baker, 2005) paradigms.

In the typical paradigm to investigate the use of property and property/kind information infants are shown with two objects differing for shape, colour or size, or for kind membership (e.g. a toy-duck and a ball), appearing one by one from one screen and disappearing behind the same screen (see Fig. 2 from Xu & Carey, 1996). In this condition, no spatiotemporal cues are available and the objects are never seen at the same time. In order to establish the number of objects in the event, infants need to rely on property/kind information, assuming that if objects do not change in their property/kind information once occluded, there should be two distinct objects behind the screen. In other words, if the infants had established representations of two distinct objects using property/kind information, they should look longer at the unexpected outcome of a single object as compared to the expected outcome of two objects. Once the screen was removed, differences in looking time showed that infants expected to see two objects in the event. This task is deemed “is-it-one-or-two” (Rips, Blok, & Newman, 2006; Rhemtulla & Xu, 2007; Xu, 2007) referring to the fact that the subject is required to determine the number of objects present in an event she/he has witnessed, such event involving two objects which differ for property/kind information.

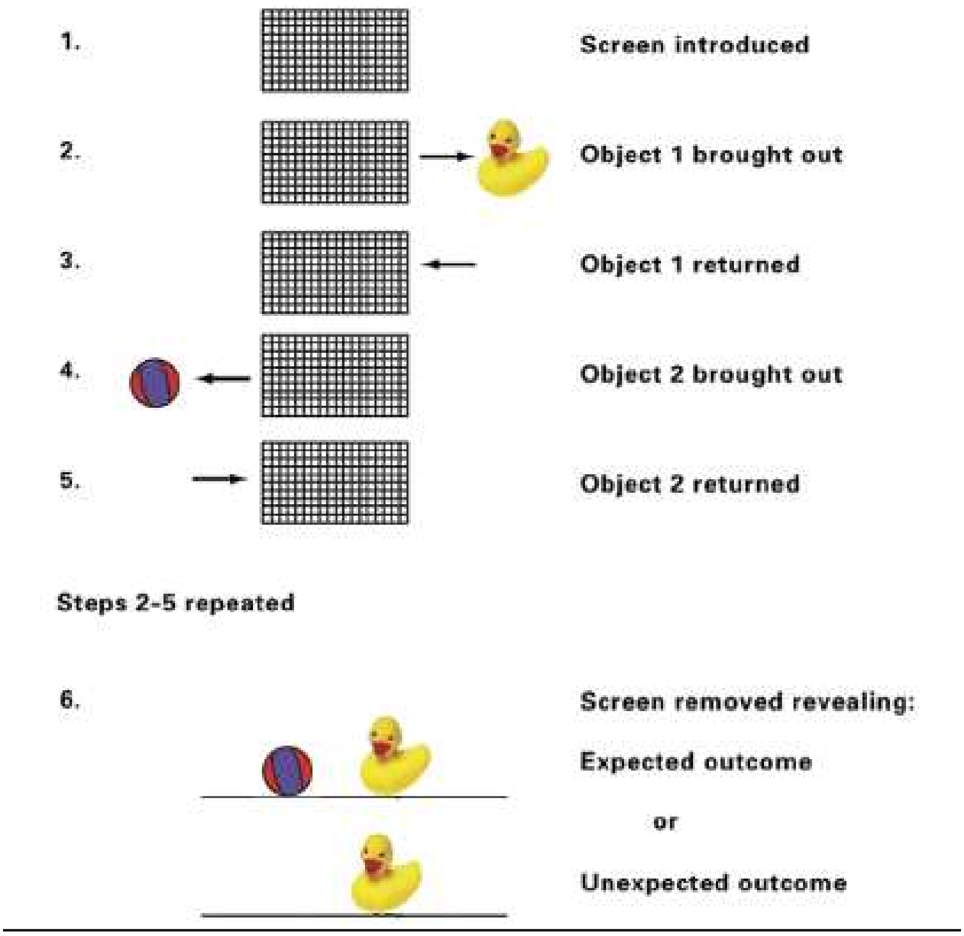


Fig. 2. Example of the looking time procedure used for investigating property/kind information (from Xu & Carey, 1996). Two featurally/kind different objects are presented one by one from behind one screen and made to disappear behind the same screen. Infants' looking times for the expected outcome of two objects and the unexpected outcome of one object were recorded.

Using this procedure it has been demonstrated that the critical age at which infants fully develop the ability to use property/kind information is 12 months and a link was hypothesized with the acquisition of language, in particular with noun understanding (Xu, 1999; Xu, 2002; Rivera & Zawaydeh, 2006). Additional studies clarified that the ability to individuate distinct objects is based on a sortal distinction between the objects, and not just on property differences (Xu, Carey, & Quint, 2004). Indeed, 12-month-old infants expected two distinct objects if the difference in shape was indicative of a sortal distinction, namely a cross-kind shape change (e.g. a cup *Vs*. a bottle of a same size, colour and surface pattern). But they did not expect two objects when the shape difference was not indicative of a sortal distinction, i.e. a within-kind shape change (e.g. a regular cup with one handle *Vs*. a sipping cup with two handles and a top lid) or if the objects differed in only colour (e.g. a red ball *Vs.* a green ball), only size or a combination of size, colour and surface pattern. This latter evidence supports the hypothesis that infants, for succeeding in this task, rely on sortal distinction because when presented with two identically-shaped objects which differ for feature, they only use the encoded information about the kind of object not considering the property differences (and, therefore, expecting that kind of object). In other words, they fail in expecting two objects when they are presented with a red ball and a green ball because they represent the sortal ball, irrespective of the different properties. Importantly, infants encoded the property differences, as revealed from longer time for habituating to successive appearances of objects with different properties than to successive appearances of identical objects. They simply failed to recruit these property differences to draw conclusions concerning the number of objects involved in the event.

On the other hand, when spatiotemporal evidence was provided, that is if objects were simultaneously visible for a few seconds, 10 months old looked longer at the unexpected outcome of a single object.

At around ten months, infants are only able to use property/kind information for establishing object numeracy when presented with animate (i.e. humanlike) *Vs.* inanimate objects (Bonatti, Zangl, & Mehler, 2002). When presented in the is-it-one-or-two task with a doll’s head and an inanimate object (e.g. pair of glued rings, toy-motorcar) infants established a mental model of two objects, looking longer if the occluder was removed to reveal just one of the two objects. Furthermore, they did not expect two objects when given a male doll's head and a female doll's head, or when given two different toy dog-heads. Thus, it seems that the infants' success is based on the sortal distinction between a person and an inanimate object. The representation of human beings develops earlier than representation of other objects.

Converging evidence was obtained employing a manual search paradigm (Van de Walle et al., 2000). Infants were presented with a box into which they could reach but could not see and they were allowed to watch the experimenter removing and then replacing objects from the box. They were tested in two conditions: in the two-object condition, two different objects repeatedly were removed and replaced from the box, one after the other; in the one-object condition, one same object was pulled out from and returned in the box twice. Infants were then allowed to reach into the box to retrieve one object. After the retrieval of the object, it was taken away and both the duration and number of subsequent reaches into the box were recorded. Unknown to the infants, in the two-object condition, the second object was removed from the box. Results showed that twelve months old infants reached longer than 10 months for the second object on the two-object condition, both in terms of reaches and duration as compare to the one-object condition. Also with this paradigm, when unambiguous spatiotemporal evidence was provided by showing both objects simultaneously, both 10- and 12-month-olds searched persistently for the missing object.

Since the seminal paper of Xu and Carey (1996), it has been argued that the acquisition of differentiate kind concepts develops with age. Infants begin with only a concept of OBJECT, undifferentiated for categories. Older infants begin to acquire different kind concepts (i.e. sortal concepts). The developmental change occurring between 10 to 12 months of age in the ability to use property/kind information was suggested to may be due to the emergence of representation of object kinds (Xu, 1999). When spatiotemporal cues are available, no object kind representation is needed. Whereas, in spatiotemporal discontinuity for establishing whether two objects, seen on different times, are one same or two different objects a specific (namely a sortal object) representation of that object is needed.

The crucial variable of language

The shift occurring between 10 months and 12 months of age has been explained with the variable of language development occurring at this age and in particular with the comprehension of the words of objects (Xu & Carey, 1996; Xu, 1999; Rivera & Zawaydeh, 2006). Xu (1999; Xu & Carey, 1996) found that infants that knew (and therefore understood) the words of an object were more successful in individuating that object. Moreover, as detailed above, 12 months old infants used kind information before of property alone: they succeeded in the is-it-one-or-two task when presented with bottles and cups before blue cups and red cups (Leslie et al., 1998; Xu et al., 2004) at the age of language acquisition. At 9 months infants succeed in the property/kind condition if both objects, when shown, were verbally referred with two (familiar) distinct labels (e.g. “Look, a Duck” or “Look, a ball”) but not when they heard the same label for both objects (e.g. “Look, a toy”). Neither the presence of two distinct tones, two distinct sounds or two distinct emotional expressions facilitated object individuation employing a violation of expectation paradigm (Xu, 2002). The role of words was extended through the investigation of object individuation using a manual search method in which objects, rather than being shown to the infants, were only labeled (Xu, Cote, & Baker, 2005). Twelve month-old infants were presented with a box (see Fig. 3). In a two-word trials the experimenter looked into the box and provided two familiar or unfamiliar labels, e.g. “Look, a car!” and “Look, a ball!” or “Look, a fep!” and “Look, a wug!”, whereas on a one-word trials the experimenter looking into the box repeated the same label, e.g. “ Look, a car!” or “Look, a zav!”. Infants were then invited to retrieve the object from the box and subsequent searching behavior was recorded. Results demonstrated that infants searched more persistently in the two-word trials as compared to the one-word trials, suggesting that hearing two labels led the infants to expect two objects. The nature of the facilitation for succeeding in this task seems to be specific to linguistic expressions. In fact, when the objects were labeled with distinct emotional vocalizations, e.g. “Aah!” and “Eew” infants did not expect two objects being in the box (Xu et al., 2005).

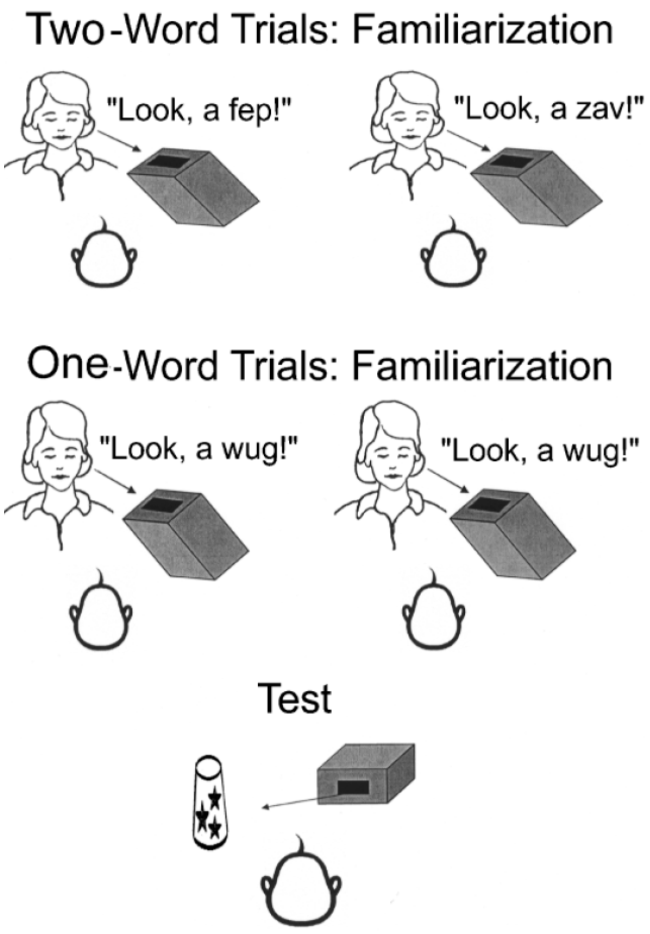


Fig. 3. Example of the manual search method in which the objects were not shown to the infants, but they were verbally labeled by the experimenter while looking inside the box (from Xu et al., 2005).

Taken together, this evidence supports the hypothesis that object individuation according to kind might be uniquely human because linguistically mediated (Xu, 2002). The facilitation observed is not due merely to the fact that labeling increases attention to objects. It seems that language, in the form of labeling, plays a specific role in signaling object kind for the infants. In conclusion, it appears that language takes a privileged part in helping infants to develop representations of object kinds (Xu, 2010).

Nonetheless, two lines of research support the independency of object individuation ability from language, namely evidence from younger infants and non-human primates.

Object individuation in younger infants

Several studies demonstrated that under certain conditions, if the cognitive demand is reduced or the event simplified, even younger infants do use featural information for object individuation (Wilcox & Baillargeon, 1998a; Wilcox & Baillargeon, 1998b; Wilcox & Chapa, 2002; Wilcox & Schweinle, 2002). This can be obtained by using a simplified version of the event mapping task or by substituting a so called *event mapping task* to an *event monitoring task*. The term *event mapping task* (Wilcox & Baillargeon, 1998a; 1998b) refers to the procedures mentioned above (e.g. Xu & Carey, 1996. See Fig. 2) in which infants, in order to establish the number of objects, firstly need to map the event consisting in successive appearances of objects from and to a screen. They then need to retrieve the representation in memory. Once the screen is removed revealing the expected or unexpected outcome, they need to compare the memory representation with the outcome shown and finally to judge whether the two are consistent. If the mapping event is simplified, by presenting a single uncomplicated trajectory or a single occlusion event, 5.5 to 9.5 months-old infants succeeded in individuating objects (Wilcox & Baillargeon, 1998a; Wilcox & Schweinle, 2002; Wilcox & Chapa, 2002).

In an *event monitoring task*, infants were shown a single event and then had to judge whether successive portions of the event were consistent: infants were shown with two featurally identical objects (e.g. two balls) or two featurally distinct objects (e.g. a ball and a box) that moved behind a screen. The screen could be either too narrow or wide enough to hide both objects simultaneously (see Fig. 4). Infants of 4.5 and 11.5 months looked reliably longer at the event in which two featurally different objects were hidden behind a narrow screen as compared to the wider screen. Whereas, when presented with two identical objects they looked about equally at narrow and wide screen events (Wilcox & Baillargeon, 1998b; Wilcox, 1999; Wilcox & Chapa, 2004). Authors interpreted the findings as reflecting infants' use of the differences between the objects to infer that two distinct objects are involved in the event: the narrow but not the wide screen event evokes longer looking because both objects could not simultaneously fit behind the narrow screen.

Moreover, converging evidence was also reported employing a simplified manual search paradigm with infants as young as 5 months of age (McCurry, Wilcox, & Woods, 2009).

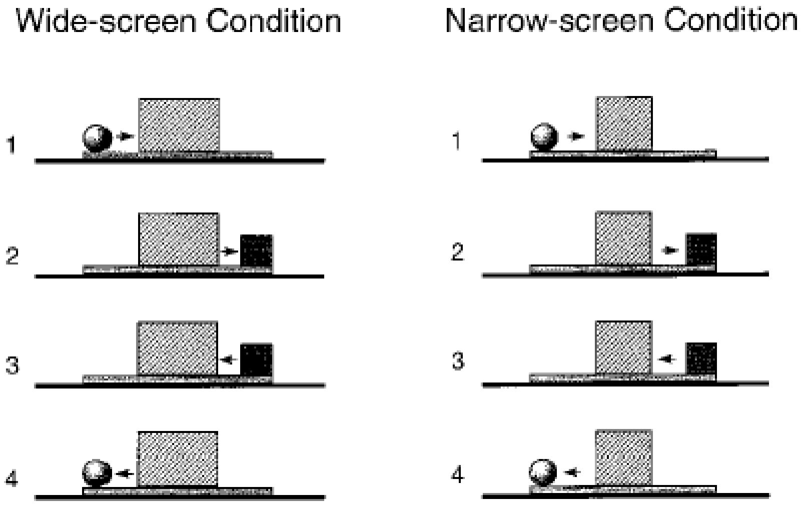


Fig. 4. Example of the *event monitoring task* (from Wilcox & Ballairgeon, 1998a). Two featurally different objects are presented from a screen. In the wide-screen condition, the screen is wide enough for hiding both objects simultaneously, whereas in the narrow-screen condition the screen is too narrow for hiding the two objects.

Analysis of differences in methods and results

The analysis of the procedure employed above highlights methodological differences that need to be discussed in order to explain age-differences.

First, the complexity of the objects seems not to be the crucial variable. Xu and Carey (2000) suggested that the objects employed in their tasks required infants to represent kind concepts, whereas tasks of Wilcox and colleagues did not. Xu and collaborators employed complex objects, such as trucks, cups, ducks and bottles whereas Wilcox and collaborators employed simple objects such as ball and box. Yet, Bonatti and colleagues (2001) replicated Xu and Carey (1996) paradigm using Wilcox and Baillargeon (1998a) objects and found results consistent with the 12 months-olds' development of the ability to use property information.

Second, the number of events in which objects emerged alternatively and repeatedly from a single location was different: in one condition the alternative presentation was between 3 and 7 times (Xu & Carey, 1996), whereas Wilcox and Baillargeon (1998a) alternated objects only once, with 2 discrete appearances of the objects (see Fig. 5 for methods' comparison). It seems that number of alternations of an object may be an important factor in predicting infant's success and failure in the task. Multiple alternations of objects may provide infants with evidence specifying a single object that changes properties while hidden. It has been argued that strong spatiotemporal evidence, suggesting that the event involves one object persisting through occlusion and changing properties, may take precedence over conflicting property information specifying two distinct objects. Thus, it seems that earlier than 12 months of age the spatiotemporal information is so strong that overrides property information; from 12 months of age, the use of property information is robust even when spatiotemporal information provides conflicting evidence (Xu & Baker, 2005). Converging proofs were found also comparing Van de Walle and collaborators' (2000) manual search procedure (i.e. displayed multiple alternations of the objects) with a simplified version which presented single alternation of the objects (Xu & Baker, 2005). The paradigm consisted in a single presentation of one object pulled out from and replaced into a box. The infant reaching into the box could find either the same object seen before or a completely different object. Then, the retrieved object was taken away and the infant was left facing the box. Infants searched more persistently (i.e. longer duration) for a second object if the one she/he had retrieved was the same as the one seen during familiarization.

Third, the cognitive demand seems not to completely explain age differences. In fact, as reported above, very young infants succeed in object individuation tasks employing the manual search paradigm which required a higher cognitive demand than the looking time method. Moreover, the results obtained by Xu and collaborators (1999) show that 10-month-old infants are not surprised when two visible different objects placed one upon the other, if grasped, moved together as well as when the two objects moved independently. This pattern of behaviour was considered as they do not represent two distinct objects (see below for details about this procedure).

In spite of the debate about different procedures, the physical nature of this kind of display offers unambiguous spatiotemporal information at significantly younger age.

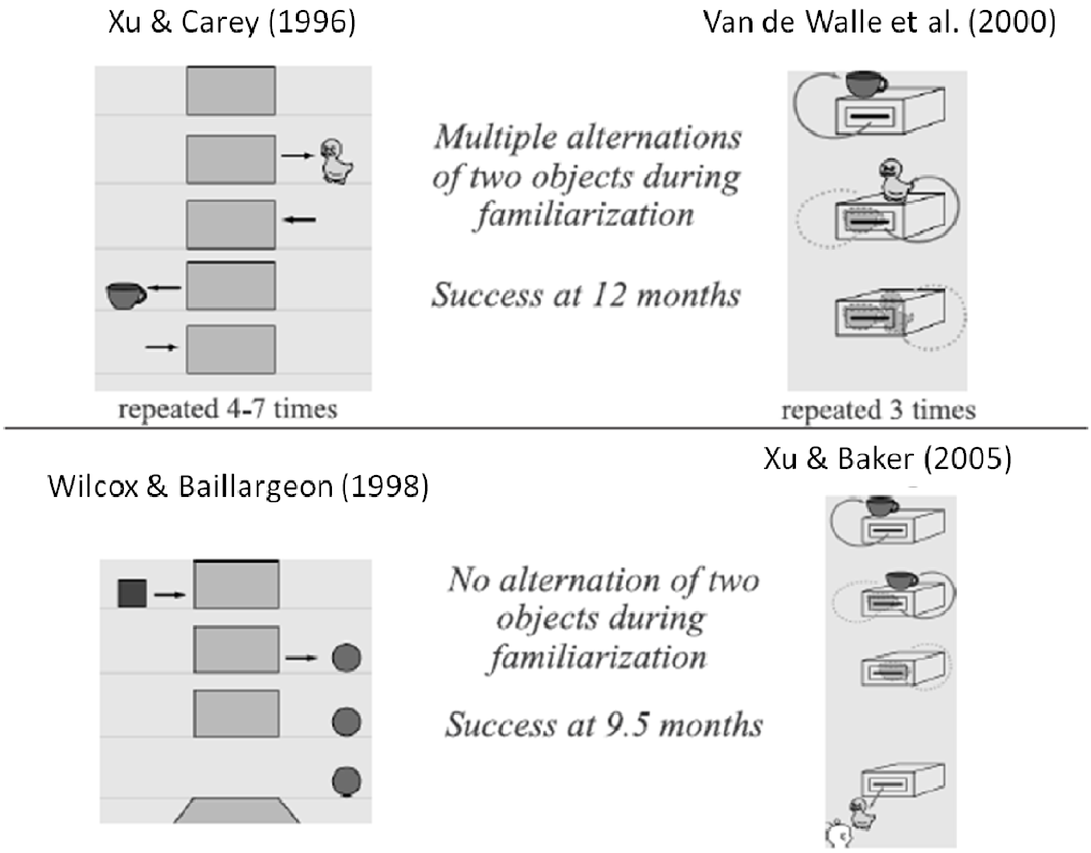


Fig. 5. Example of the procedure employed in Xu and Carey (1996) and Van de Walle, Carey and Prevor (2000) in which multiple alternations of the objects were presented and infants succeeded in individuating two objects at 12 months of age; and Wilcox and Baillargeon (1998a) and Xu and Baker (2005) in which the two objects were presented only once each. Infants succeeded at 9.5 months. Studies displayed on the left used looking time method and manual search method on the right.

Object individuation in non-verbal animals

In spite of evidence supporting the role of language, comparative researches have proved that some non-human animal species are able to use all of the above described types of information for object individuation. The process of parsing the visual information into distinct objects and forming mental representations of those objects is ecologically relevant to animal survival in the natural environment: to avoid obstacles, find food, detect predators and conspecifics.

Munakata, Santos, Spelke, Hauser and O'Reilly (2001) demonstrated that monkeys parse array of adjacent food items into distinct objects, representing those objects as separately movable whereas it is only at around 12 months of age that this ability is available in infants (Xu et al. 1999, to be noted that the cognitive demand required for this task is reduced; it is not necessary to memorize successive display of the event for succeeding, see Fig. 6). In this paradigm subjects were habituated with two objects, different for property/kind, one on top of the other (e.g. a pumpkin on top of a pepper for monkeys; a toy-duck on top of a toy-car for infants). In one familiarization condition, the objects were stationary, in the other the top object was moved relative to the bottom object. During test condition, a hand grasped the top object and lifted it. Two events were presented: in one the top object rose into the air while the bottom remained on the supporting surface, in the other, both objects moved upward together. Both monkeys and 12-months-old infants looked longer at the event in which the two adjacent objects moved as one unit than at the event in which they moved separately, demonstrating to represent the two objects as distinct. Ten-month-old infants represented two objects, showing the same looking pattern, only in the condition in which objects were initially presented in motion and not when they were initially stationary. These findings provide further evidence that, in humans, the ability to use spatiotemporal information is available earlier than the ability to use property/kind information.

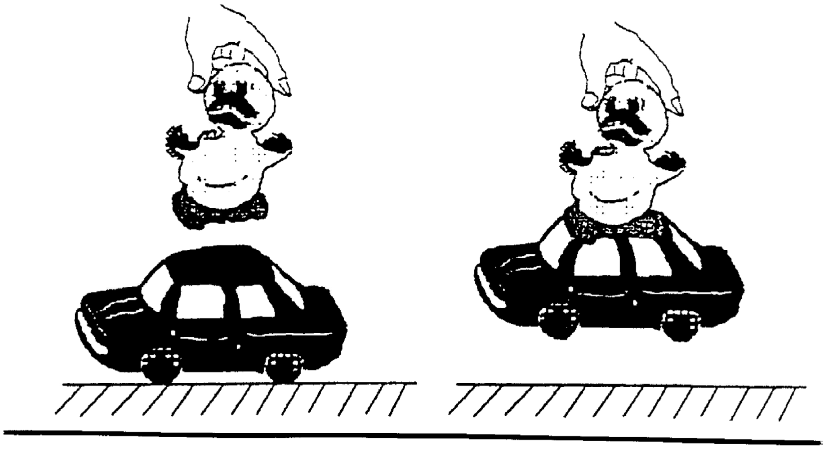


Fig. 6. Example of the procedure used in Xu, Carey and Welch (1999).

Adult non-human primates (namely, monkeys and great apes) are able to use spatiotemporal as well as property/kind information for individuating objects employing the is-it-one-or-two task, both with looking time measures and manual search paradigms (Uller, Xu, Carey, & Hauser, 1997; Santos, Sulkowsky, & Hauser, 2002; Phillips & Santos, 2007; Mendes, Rakoczy, & Call, 2008; Mendes, Rakoczy & Call, 2011). In particular, using a modified version of Xu and Carey (1996) habituation methodology, Uller and colleagues (1997) demonstrated that rhesus monkeys (*Macaca mulatta*) succeeded in individuating objects both with spatiotemporal and property/kind information. Subjects were presented with an event in which a carrot was removed from and then replaced behind a screen, followed by a piece of squash removed from and replaced behind the same screen. Once the screen was lifted, the expected event of a carrot and a squash or the unexpected event of only one of these objects was presented. Monkeys looked longer at the latter event demonstrating to expect two objects being involved in the event.

This evidence was extended using a modified version of manual search paradigm used by Van de Walle and colleagues (2000). Monkeys who had at first seen one piece of food being placed inside a box, when allowed to search for it in the box, could find either that same food (consistent condition) or a different food (violation condition), which had been surreptitiously placed there by the experimenter. Monkeys detected the change in property, searching longer in the violation condition as compared to the consistent condition (Santos et al., 2002). These abilities are shared also by great apes (Mendes et al. 2008; 2011), such as chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*) and gorillas (*Gorilla gorilla*).

An objection against the ability of representing kinds without language, and therefore, against those results, claimed that monkeys could solve that problem without using kind representation but relying on property information (Xu, 2002). In fact, in all of the studies reported above, property and kind information are confounded: different kind objects involved as stimuli were perceptually different from each other. Interestingly, those features co-vary even in the infants' studies but, as already stressed, infants encode kind differences before property differences demonstrating to possess sortal concepts of that objects.

In order to shed light on the question regarding the role of language for kind representation, Phillips and Santos (2007) provided evidence that rhesus monkeys successfully individuated different kind of objects even when their perceptual information was held constant. In particular, the experimenter showed to the monkeys a familiar fruit, for example a coconut and a small piece of that fruit (which actually was a piece of white plastic perceptually identical to the fruit) was visibly placed inside the box (see Fig. 7). Subjects were let free to retrieve the fruit from the box. In the testing trials, the animals found either a consistent (e.g. a piece of coconut) or an inconsistent kind of fruit (e.g. a piece of apple). Because all the subjects saw the same perceptual object entering the box, differences in reaching times scored for the two conditions suggested that monkeys expected a particular kind of fruit which varied in kind but not in perceptual properties, searching longer in the inconsistent condition.

This research demonstrated that monkeys possess some sort of long-term representation of at least two kinds of objects, which supplies in individuating object through non-visible properties (which constitute the represented kind).

Fig. 7. Example of the procedure employed in Phillips and Santos (2007) task in which rhesus monkeys were presented with kind different but featurally identical objects.

Recently, object individuation ability has been proved also in another mammalian species, such as domestic dogs (*Canis familiaris* Bräuer & Call, 2011). The authors compared great apes and dogs performance using a violation of expectation paradigm in an identical task. They presented a “magic cup”, a container with a double bottom that allowed to change the type of food subjects discovered inside. The animals were provided with either good or bad food items placed inside the magic cup and then, they could find same or different kind of food which was changed by the experimenter. Dependent variables considered were the number of trials in which apes looked inside the box and dogs smelled the cup (within 10 seconds of recording). Results demonstrated that both species were individuating a particular kind of food. In fact, both great apes and dogs reacted both to negative (i.e. good food substituted for bad food) and positive (i.e. bad food substituted for good food) surprises; moreover, they reacted more often to the negative surprises (i.e. higher number of trials in which they looked or smelled). Thus, it seems that apes and dogs are capable of individuating objects according to their property/kind information, showing similar behavioural patterns.

Evidence carried out with those non-verbal mammalian models, therefore, supports the idea that object representation according to kind is independent from language.

The crucial variable of previous experience

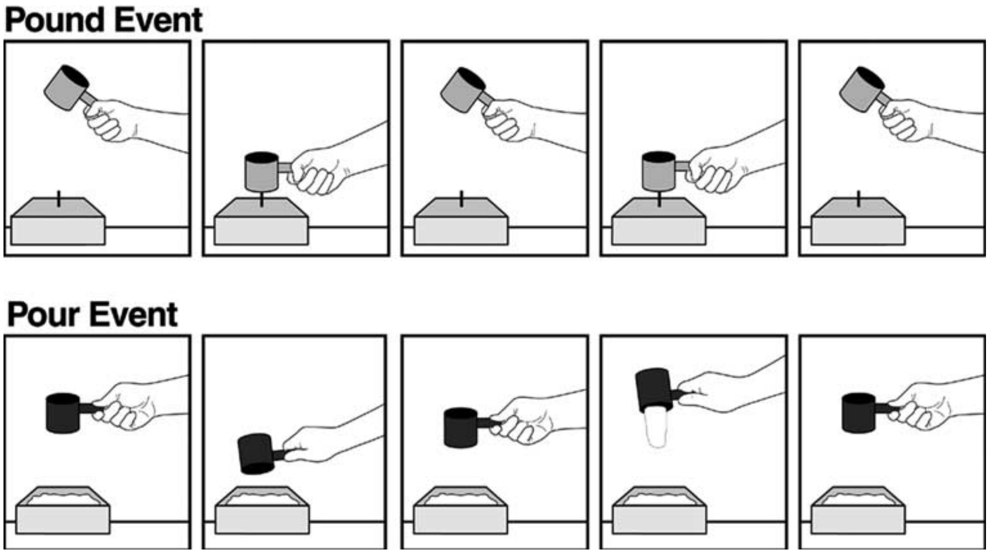
Another crucial variable seems to be the experience with the objects.

Infants’ previous experience with the physical world would play a critical role in their ability to use featural information in object individuation. In particular, a specific experience with the objects is crucial for using property information provided by the colour. This occurs when the pattern or the colour of an object is experienced as being predictive of its function (Wilcox & Chapa 2004; Wilcox, Woods, & Chapa, 2008), or when infants are given multisensory exploration of the objects (Wilcox, Woods, Chapa, & McCurry, 2007).

Infants from 4.5 months use shape or size features for individuating whether two objects are involved in an event but it is not until 7.5 months that they use pattern information and only at 11.5 months successfully use colour information (Wilcox, 1999). The critical age decreases at 4.5 months for pattern and 7.5 months for colour if infants are previously exposed to events in which the pattern or the colour of an object is experienced as predictive of its function (colour-function condition; Wilcox & Chapa, 2004). In a pre-test condition, infants were shown a pair of events in which two shaped identical objects which differed from each other for colour, each carried out a distinct function. In the pound event, the green object pounded a wooden peg; in the pour event, the red shape identical object scooped and poured salt (see Fig. 8a). The shape of the objects was manipulated in the two or three pre-test events, maintaining both the colour and the function constant. In this way, infants learned to associate only colour with function. At test, they were shown with a green and a red ball in the narrow and wide screen test (i.e. the event monitoring task of Wilcox, 1999. See Fig. 8b). Results demonstrated that they attended to colour information for individuating objects, only when the colour was predictive of the function the object would engage in. The movement itself of the object, namely presenting different coloured objects moving without carrying out a function, did not increase the sensitivity to colour. Moreover, the sensitivity to the functional properties of objects increases and, therefore, the age development decreases according to the increasing of the number of pre-test events. However, the ability to generalize to other colours emerges at 9.5 months if infants are experience with multiple pairs of colours in the colour-function condition (Wilcox et al., 2008).

Differently, monkeys spontaneously used the information provided by colour differences to individuate objects of the same kind in the domain of food (Santos et al., 2002) but they can use shape differences only after some previous experience. Only few encounters with shape-different food prior to the test trials were enough for monkeys' succeeding in this task (Mendes et al., 2011).

a)



b)

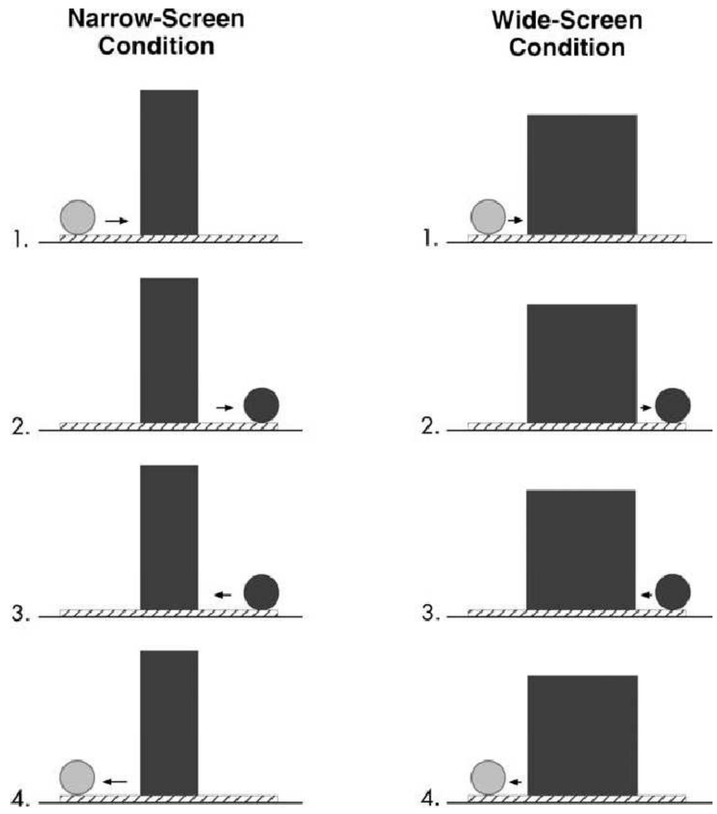


Fig. 8. Representation of Wilcox and Chapa's (2004) colour-function experiments.
a) Schematic representation of the pound and pour pretest events of Wilcox and Chapa (2004). The container used in the pound event was green and the identical featural container used in the pound event was red. b) Representation of the test events in the narrow and wide-screen conditions. A red and a green ball were presented.

THE PRESENT RESEARCH

To date, no evidence is available for non-mammalian animal species, neither for young individuals. In fact, studies carried out with non-human primates and dogs focused on adult animals.

Therefore, the present research represents the first attempt to investigate object individuation in a vertebrate species, the domestic chicken (*Gallus gallus*), phylogenetically distant from mammals. The use of an avian model, endowed with a neural architecture much different from the mammalian one, allows for inferences concerning the remote origin of this ability. Indeed, object individuation ability is possibly shared by the two animal classes and inherited from a common ancestor, as lineages of the Amniote group, such as birds and mammals, separated about 300 million years ago from the ancestral stem reptiles (Rogers & Andrew, 2002).

Moreover, this bird species permits to test very young individuals which allow to finally establish whether this ability can be early available during development and independent from experience.

The model

The animal model employed offers several advantages for behavioural experiments.

Domestic chicken (*Gallus gallus*) is a highly visual species: it is endowed with acute sense of colours (Osorio, Vorobyev, & Jones, 1999) and shares with other vertebrate species the properties used to represent objects by the perceptual experience (for instance see Regolin & Vallortigara, 1995; Regolin et al., 2011).

The chick is a precocial species: it hatches with a brain ready to learn critical information for survival, possessing a sensory-motor behaviour that is virtually identical to that of the adult animal, and it can independently feed almost immediately. This allows to test animals only few days or even hours after hatching. Another relevant feature is the perceptual learning involved in filial imprinting. Filial imprinting is the process whereby young birds of a precocial species learn to recognize the first conspicuous object they are shortly exposed to soon after hatching (Lorenz, 1935; Bateson, 1966). During the period of exposure to the visual stimulus the chick rapidly forms a memory of its features and develops a strong social attachment to it. Usually, the natural imprinting stimulus is the mother hen, but artificial objects proved to be equally successful in triggering social attachment and social bonding in young chicks, with moving stimuli preferred over stationary ones (e.g. Bateson, 1966; Bolhuis, 1991; Horn, 1985; see for a general review Bolhius & Honey, 1998; Bateson, 2000). Therefore, learning through imprinting proved to be a more ecological technique than the traditional conditioning procedures to investigate perceptual and cognitive phenomena in precocial birds' species (for instance see Regolin & Vallortigara, 1995). Finally, the possibility to nearly completely control for environmental/experiential factors both during incubation and after hatching allows to investigate almost innate ability and it turns to be an excellent model system for the investigation of behavioural neurobiology (Andrew, 1991; Rose, 2000). The use of the chick has also practical advantages: it is an animal easy to obtain, little expensive, and its neuroanatomy and neurophysiology are very well known with respect to other species. Moreover, chicks' behavioural responses are discrete and easy to detect, making the behavioural measures more objective.

Finally, the domestic chicks have been already demonstrated to possess several core knowledge systems shared with human infants (see for a review Vallortigara, Regolin, Chiandetti, & Rugani, 2010): the system of social partner representations (Rosa-Salva, Farroni, Regolin, Vallortigara, & Johnson, 2010); the system for representing agents and their actions (Vallortigara, Regolin, & Marconato, 2005; Vallortigara & Regolin, 2006; Mascalzoni, Regolin, & Vallortigara, 2010); the core system for number representation Rugani, Regolin, & Vallortigara, 2007; Rugani, Regolin, & Vallortigara, 2008; Rugani, Fontanari, Simoni, Regolin, & Vallortigara, 2009; Rugani, Regolin, & Vallortigara, 2010); the system for representing space (Chiandetti, Regolin, Sovrano, & Vallortigara, 2007; Chiandetti & Vallortigara, 2008a; Chiandetti & Vallortigara, 2008b).

Aims

The present research aims at investigating the information used by two/three days old chicks in object individuation task, allowing to finally establish whether this ability can be available early in development and independent from experience and from language.

Data combined with that obtained with non-verbal species would complement and extend evidence from pre-verbal infants, as the latter do not completely rule out the role of language predisposition, which is inherent in our species. Moreover, it would extend evidence obtained with non-verbal mammals controlling for the role of experience.

More broadly, this research contributes to the understanding of the foundations of object representation, which constitute one of the basic functional units hypothesized by the core knowledge theory (see Spelke & Kinzler, 2007; Carey, 2009; for a review on comparative data see Vallortigara et al., 2010).

To the purpose of studying object individuation ability, the use of property information, spatiotemporal information and property/kind information had been addressed through an experimental design based on a task inspired to the event-mapping task of Xu and Carey (1996). Filial imprinting onto artificial objects was exploited as a behavioural tool: once imprinted, chicks spontaneously tend to stay as close as possible to the imprinting object. Therefore, if the object visibly moves away, chicks promptly and closely follow it. This natural following response was employed for motivating chicks to perform behavioural choices. Moreover, since an effect of gender has been described on behavioural investigations in domestic chicks, only female chicks were selectively tested to reduce variability. Female chicks display stronger social reinstatement tendencies following filial imprinting than male chicks, and this has been attributed to stronger social motivation in females (Vallortigara, 1992; Cailotto, Vallortigara, & Zanforlin, 1989; Vallortigara, Cailotto, & Zanforlin, 1990; Jones & Williams, 1992).

I took advantages also from other chicks' capabilities which were employed as behavioral tools in this research: chicks' ability to rejoining hidden imprinting objects by walking towards the location where the object was seen disappearing, remembering the position up to 180 seconds from its disappearance (Regolin, Garzotto, Rugani, Pagni, & Vallortigara 2005; see also Vallortigara, Regolin, Rigoni, & Zanforlin, 1998; Vallortigara, 2000; Regolin, Rugani, Pagni, & Vallortigara, 2005); chick's ability to spontaneously discriminate between different quantities of both imprinting objects or food items (Rugani et al., 2009; Rugani et al., 2010; Regolin, Rugani, & Vallortigara, 2010). When chicks are placed between two visible groups of different quantity of imprinting objects they spontaneously prefer the larger quantity of familiar objects (Rugani et al., 2010). Moreover, they are able to spontaneously discriminate between groups of different number of objects (e.g. 3 and 2 objects) which were made to disappear, one after the other, each behind one of two identical opaque screens (see Fig. 9). Once chicks are set free to choose between the two screens they show to prefer the larger group of objects (Rugani et al., 2009).

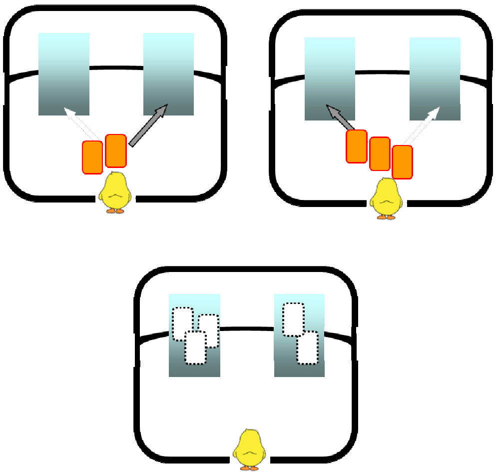


Fig. 9. Schematic representation of chicks' discrimination task from Rugani, Fontanari, Simoni, Regolin and Vallortigara (2009). Two and three objects were shown to be hidden, one by one, behind two opaque screens.

In order to explore property and spatiotemporal information ability, sets of two-dimensional imprinting objects were employed, which allowed to easily manipulate or holding constant several features. In particular, the property information was provided by colour, size, shape and peculiar feature (i.e. different orientation of two black segments on each stimulus) of the objects. Chicks were exposed to those sets of objects for the first days of life, so that towards the process of filial imprinting they developed a social attachment becoming motivated to rejoin them.

Furthermore, in order to test property/kind information, a consideration about what category of objects chicks should possess at birth is needed. Because of being domestic chicks precocial birds, able to follow the mother hen and the siblings and to feed independently soon after birth, it should be plausible that they precociously exhibit some competences concerning the categories of objects most crucial to their survival. Rudimental representations of object categories may in fact prove useful for an animal dealing with its own environment and may therefore result in an increased fitness.

Feeding and social behaviour certainly constitute the two main domains in the chick's early life. Both categories are crucial to the newborn's survival, though they imply very different behavioural responses, and plausibly different levels of motivation are associated with either domain under differential environmental conditions. Thus, food and social stimuli were employed in the object individuation task, after having established rearing and testing conditions in which chicks seemed to be equally motivated to those attractors.

A free choice paradigm with a delayed response task was designed for investigating object individuation. Chicks were placed in a circular arena with two identical opaque screens. An equal number of groups of events was presented by each screen. The groups of events could be either a simultaneous presentation or a sequential presentation of the stimuli, accordingly to the experimental conditions. An event was the presentation of one stimulus.

In the Simultaneous Presentation test, two events were sequentially presented, each event consisted in the simultaneous presentation of two (or three) objects which were made simultaneously appearing from one screen, coming in front of the chick, temporarily confined in the holding box, and then made to slowly disappear behind the same screen.

In the Sequential Presentation test each chick was shown two groups of events, each taking place by one of the two screens only. One group of events consisted in two (or three) presentations of one same stimulus. The other consisted in a single presentation of two different stimuli. In this way, an equal numbers of events were shown to the chick, each group of events taking place in a different spatial location. The two groups of events differed in the actual number of objects involved (either one or two).

The prediction would be that, if chicks spontaneously approach the larger quantity of imprinting objects they would choose the screen that hid two objects, even though they have seen an equal number of events. This would determine whether they are able to establish the number of objects presented using the information provided by the properties or kinds of the objects, irrespectively of the number of presentations witnessed.

This briefly described paradigm was employed for investigating chicks' ability to use spatiotemporal information, property information and property/kind information for object individuation in two or three day old chicks.

GENERAL MATERIALS AND METHODS

SUBJECTS AND REARING CONDITIONS

Subjects were 188 female ‘Hybro’ (a local variety derived from the White Leghorn breed) domestic chicks (*Gallus gallus*), obtained weekly from a local commercial hatchery (Agricola Berica, Montegalda, Vicenza, Italy) when they were only a few hours old. On their arrival at the laboratory, chicks were immediately housed in standard metal home cages (28 cm wide x 32 cm long x 40 cm high) at controlled temperature (28–31°C) and humidity (68%), with food and water available *ad libitum* in transparent glass jars (5 cm in diameter, 5 cm high) placed at each corner of the home cage. The cages were constantly (24 h/day) lit by fluorescent lamps (36 W), located 45 cm above the floor of the cages. Each chick was placed singly in one cage, together with a set of objects (which were different on the basis of the experimental condition, see each experiment for details), which constituted its imprinting stimulus. Each object was suspended in the centre of the cage by a fine thread, at approximately 4–5 cm from the floor and 2 cm from the other objects, so it was located at about chicks’ head height at a distance that allows to individually familiarize with each object.

APPARATUS

Training and testing took place in an experimental room, located near the rearing room, in which temperature and humidity were maintained, respectively, at 25°C and 70%. The room was kept dark, except for the light coming from a 40 W lamp placed approximately 80 cm above the centre of the apparatus. The testing apparatus (Fig. 10) consisted in a circular arena (95 cm in diameter and 30 cm outer wall height) with the floor uniformly lined with a white plastic sheet. Within the arena, adjacent to the outer wall, there was a holding box (10 x 20 x 20 cm), where the chick was confined shortly before the beginning of each trial. The box was made of opaque plastic sheets, with an open top allowing the insertion of the chick before each trial. The side of the holding box, facing the centre of the arena, consisted of a removable transparent partition (20 x 10 cm), in such a way that the subjects, while confined, could see the centre of the arena. During the training phase, one single cardboard screen (16 x 8 cm) was used, positioned in the centre of the arena, in front of and 35 cm away from the holding box. During testing, two opaque cardboard screens, identical in size, colour and pattern (i.e. blue coloured with a yellow 'X' on them) to the one used during training, were positioned in the centre of the arena (see Fig. 10), symmetrically with respect to the front of the holding box (i.e. 35 cm away from it and 20 cm apart from one another).

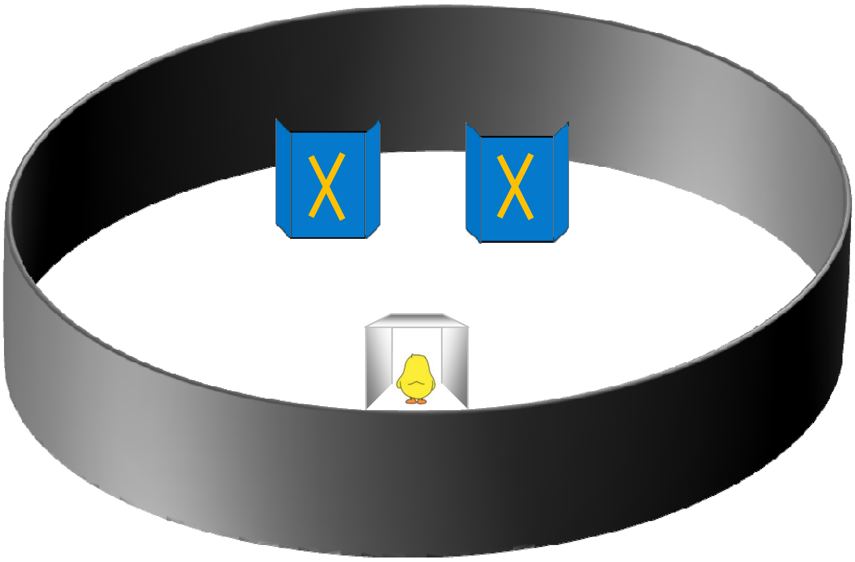


Fig. 10. The apparatus used in the testing phase of all the Experiments (in the training phase only one of the two screens was present in the centre of the enclosure).

PROCEDURE

Training

On day 2 or 3 (see each experiment for details), chicks underwent a preliminary training session. Each chick was placed within the testing arena (not confined in the holding box), in front of one screen, together with a single visible stimulus. The stimulus was identical to one of the stimuli used during rearing (which type of stimulus was chosen was randomized across subjects).

The stimulus was held from above by the experimenter (not visible to the chick), via a fine thread, and initially kept suspended between the holding box and the screen. The chick was left free to move around and get acquainted with the environment for approximately five minutes. Thereafter, the experimenter slowly moved the stimulus towards the screen, and then behind of it, until the stimulus disappeared completely from chick's sight. This procedure was repeated a few times, until the chick responded by promptly following and rejoining the stimulus behind the screen. Thereafter, the chick was confined within the holding box, behind a transparent frontal partition through which it could see the stimulus being moved and hidden behind the screen. As soon as the stimulus had completely disappeared from sight, the chick was set free in the arena by lifting the transparent partition. Every time the chick rejoined the stimulus, as a reward, it was allowed to spend a few seconds close to it. The whole procedure was restarted and the training ended when the chick had rejoined the stimulus for three consecutive times. On average, to complete the training approximately 15 minutes were required for each chick.

Testing

One hour after training, the chick underwent testing. At the beginning of each testing trial, the chick was confined within the holding box, behind the transparent partition, from where it could see the two identical screens in the centre of the arena. Before starting with the presentation of the stimuli in each trial, all of the stimuli to be involved in a same group of events were positioned behind the same screen; during this manipulation phase an opaque partition (made of a white paper sheet) was temporarily placed in front of the holding box in order to prevent chick's sight. The opaque partition was then removed and the trial could start.

Two testing procedures were employed for different samples of subjects: Sequential Presentation test and Simultaneous Presentation test.

In the Sequential Presentation test each chick was shown two groups of events, each taking place by one of the two screens only. One group of events consisted in two presentations of one same stimulus (identical to the stimulus employed during training). Exceptions in the number of presentations of the stimuli were made in Experiment 4 and Experiment 5 in which the stimulus was presented three times (see each experiment for details), while in Experiment 6 stimuli presented at test were of new colours as compared to the imprinting stimuli. The presentation of this group of events was as follow: the stimulus was made to appear from behind the screen where it had been previously positioned, it was slowly moved towards the front of the confined chick, it remained in front of the chick (beyond the clear partition) for a few seconds, and then it was made to disappear behind the same screen from where it had initially come from, and it was left behind of it. It was then picked up once again and the whole procedure was repeated so that the same stimulus was shown twice to the chick.

The other group of events consisted in a single presentation of two different stimuli (each identical to one of objects of either sub-groups used for imprinting, except for Exp. 6). The same stimuli presentation's procedure was carried out for this other group of events, with the only difference that in this case two different stimuli were sequentially presented, once each, to the chick. In this way, an equal number of events (i.e., two) took place by each screen, differing in the actual number of objects involved (either one or two).

The whole procedure lasted between 40 and 45 seconds. Each stimulus took three seconds for being moved from behind the screen to the front of the holding box, where it was kept for another three seconds, and then it took three seconds to be moved back behind the screen (nine seconds overall). About two seconds elapsed from the disappearance of one object and the appearance of the next (either same or different) object.

In the Simultaneous Presentation test, two events were sequentially presented, each event consisted in the simultaneous presentation of two (or three in Exp. 3) objects: either two (or three) identical or two different objects (except for Exp. 7 and Exp. 8 in which two identical imprinting stimuli *Vs.* two identical food stimuli were presented). In each event, each set (comprising two or three objects) was made simultaneously appearing from one screen, coming in front of the chick confined in the holding box and then made to slowly disappear behind the same screen. The whole procedure took approximately 20-25 seconds: each group took three seconds for being moved from behind the screen to the front of the holding box, where it was kept for another three seconds, and then it took three seconds to be moved back behind the screen (nine seconds overall) and about two seconds elapsed from the disappearance of one and the appearance of the next group.

In both tests, immediately after the disappearance of both sets of objects (with a delay of five seconds), the transparent partition was removed and the chick was left free to move around and search for the stimuli within the arena. To prevent the chicks from spotting the hidden stimuli before having circled almost completely around of the screen, the two vertical sides were provided with 3 cm bent back edges.

A choice was defined as when chick's head had entered the area behind one of the two screens enclosed by the edges of the screen. Only the choice for the first screen visited was scored and thereafter the trial was considered over. The response time, i.e., the time (seconds) taken by the chick to detour the chosen screen was also scored and analyzed. At the end of each trial, chicks were allowed to spend one-two seconds together with their 'social companions' present behind the screen (and in Exp. 7, Exp. 8, Exp. 9, Exp. 10 they were allowed to eat food items) that had been chosen. If a chick did not approach either screen within three minutes, the trial was considered null and void and it was repeated immediately afterwards. After three consecutive null trials, the chick was placed back within its own rearing cage (in the presence of the imprinting objects) for approximately one hour before being resubmitted to further trials. After another three consecutive null trials, the same procedure was repeated and if, once again, the chick scored for the third time three consecutive null trials, it was discarded from the experiment (this occurred for about 5% of chicks, not included in the final sample).

Each chick underwent one complete testing session of ten valid trials. The order of presentation (which group of events was first and which second) of the two groups of events (e.g. one same stimulus presented twice *Vs.* two different stimuli presented once each) as well as the position (left screen – right screen) of appearance and disappearance of either set was counterbalanced within each chick's ten testing trials. Stimuli employed in the two group of events were randomised between subjects, whereas the order of the events as well as which screen concealed which event were counterbalanced within each chick's ten testing trials.

For both the Sequential Presentation test and the Simultaneous Presentation test, the number of trials in which each chick detoured the screen hiding two different stimuli was considered and regarded as the correct choice (except for Exp. 3, in which choice for the screen hiding three identical stimuli was regarded as the correct choice).

The behaviour of chicks was entirely video-recorded through an overhead video camera. Chicks' choices were scored both on-line by direct observation by an experienced observer and then, off-line, by a blind observer. The two scoring systems were highly consistent with each other.

Percentages of responses were computed as: (number of correct choices/10) x 100.

PROPERTY and SPATIOTEMPORAL INFORMATION

This first series of experiments aimed at studying chicks' ability to use property as well as spatiotemporal information. To this purpose, the paradigm designed employed, as imprinting stimuli, two-dimensional artificial objects that allow to easily manipulate features, such as size, shape (and volume) consistently with the goal of the experiment. There is an extensive literature on actual effectiveness of artificial objects in triggering social attachment and social bonding in young chicks through the process of filial imprinting (e.g. Bateson, 1966; Bolhuis, 1991; Horn 1985; see for a general review Bolhius & Honey 1998; Bateson, 2000).

A further advantage of using social stimuli is that chicks are not reinforced with food. This allows to better control for motivational factors (i.e., if chicks are food-reinforced, at each test trial, motivation for food can decrease, as the birds become satiated. See the second series of experiments for considerations about this issue).

<u>EXPERIMENT 1</u>

The first experiment investigated whether chicks make use of property information provided by Colour, Size or Shape for object individuation.

Chicks were tested in a 2 *Vs.* 2 events comparison (i.e., one object presented twice *Vs*. two different objects each presented once). Previous studies demonstrated that chicks approach the larger group of imprinting objects (Rugani et al., 2009; 2010). Therefore, if chicks use property information for individuating objects, they should approach the group of events featuring two different objects; if chicks do not use such information, they should behave at random, as they are faced with a choice between two sets comprising a same number of events.

MATERIALS and METHODS

SUBJECTS

Subjects were 41 female domestic chicks (*Gallus gallus*) reared in accordance to the general rearing conditions. Separate groups of chicks took part to Colour condition (N=12), Size condition (N=12) or Shape condition (N=17): they were reared and tested with different types of property information available.

STIMULI

The stimuli for the Colour condition were three green squares and three yellow squares (4 x 4 cm); the stimuli for the Size condition were three yellow squares (4 x 4 cm) and three smaller yellow squares (2.77 x 2.77 cm); the stimuli for the Shape condition were 3 yellow squares (again, 4 x 4 cm) and three yellow “Greek crosses” (4 x 4 cm axis long, obtained from a 4 x 4 cm square following removal of a square area of 1.33 x 1.33 cm from each corner). Within each experimental condition, the same stimuli were used for imprinting, training and test (Fig. 11). The stimuli were suspended in the home cage at about 2 cm from each other, and overall they occupied an area of about 8 x 8 cm in the centre of the cage at a distance that allows to individually familiarize with each object. The imprinting objects were made of uniformly coloured cardboard covered with a transparent plastic film. Each set of six objects was composed of two sub-sets made of three identical objects each. The two sub-sets differed for either colour, size or shape depending on the experimental condition.

Chicks were kept in the standard rearing conditions described above from the morning (11.00) of the first day (i.e. Monday, the day of their arrival, which was considered as day 1) to the third day (Wednesday). In the morning (11.00) of day 3, chicks underwent the training, and approximately one hour later, they took part in the first testing session.

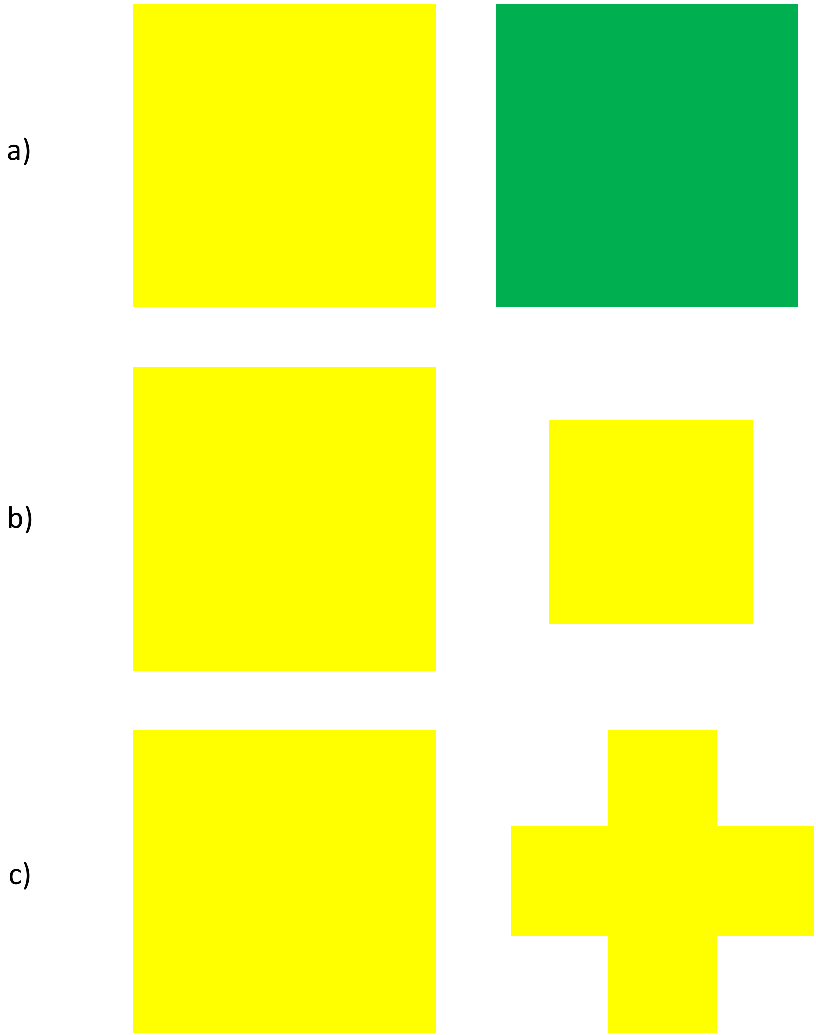


Fig. 11. The two types of objects used in Experiment 1 for imprinting, training and test in each of the three experimental conditions: a) Colour; b) Size; c) Shape.

PROCEDURE

Training

On day 3, in the morning, chicks underwent a preliminary training session. The stimulus was identical to one of the six stimuli used during rearing (the type of stimulus chosen was randomized across subjects). An exception was made in the Size condition, where the larger yellow square was used at training for all subjects (experience with the larger stimulus needed to be equalized in this condition as such stimulus may constitute a supernormal stimulus for the chicks; Tinbergen, 1948). The training procedure was identical to the general training procedure described above.

Testing

The testing procedure is conformed to the Sequential Presentation test in which the chick was shown a single presentation of two stimuli differing for property information (each identical to one of the two sub-groups of objects used for imprinting, and the two stimuli at test differed for one property i.e., Colour, Size or Shape) sequentially presented *Vs*. one same stimulus presented twice (identical to the stimulus employed during training), see Fig. 12.

The number of trials in which each chick chose the screen hiding two stimuli differing for one property (which was regarded as the correct choice) was considered and percentages were computed as: (number of correct choices/10) x 100. Analysis of variance (ANOVA, to compare the percentages of correct choices between the three different experimental conditions based on property information provided by Colour, Size and Shape) and one-sample two-tailed t-test (to assess significant departures from chance level, i.e. 50%) were used. Non-parametric statistics was performed employing the chi-square test for analysing performance in the chick's initial trials. A repeated measures ANOVA was computed on the response times (seconds) as dependent variable, scored for "correct" *Vs*. "non-correct" trials (repeated measures variable).

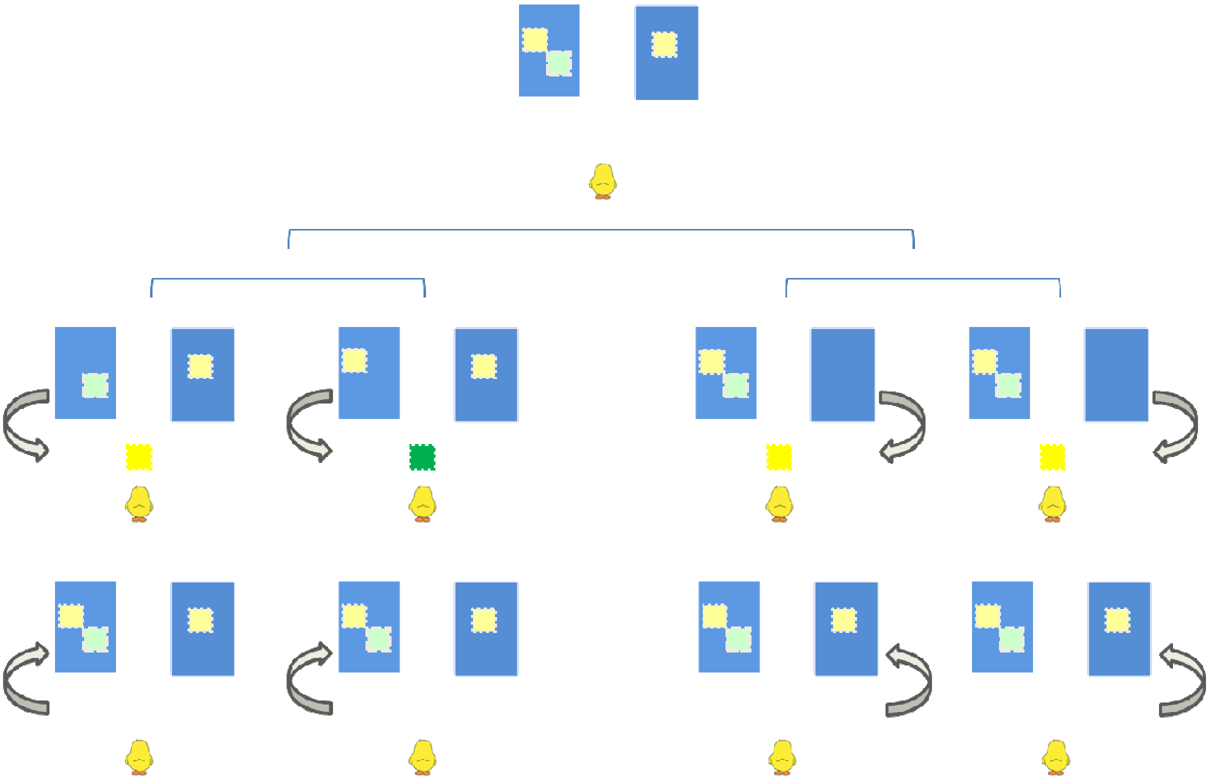


Fig. 12. Example of presentation of the stimuli in the Sequential Presentation test (Colour condition). The first group of events used a single presentation of two stimuli (on the left side). The second group of events employed the same stimulus twice (on the right side).

RESULTS AND DISCUSSION

No statistically significant main effect of the type of property information ($F_{(2,38)}$= 0.133; p=0.876) was revealed by the ANOVA run on the percentages of correct choices (Colour N=12, mean= 59.166; sem= 3.362; Size N=12, mean= 60.833; sem= 2.289; Shape N=17, mean= 61.176; sem= 2.829). Data were therefore merged and the overall mean was compared with chance (50%) through a one sample t-test. Chicks preferentially approached the screen hiding two different objects (t(40)= 6.410; p<0.001; mean = 60.487; sem= 1.636; see Fig. 14 – Exp. 1 in which only colour condition is displayed because it was arbitrarily chosen for carrying out following experiments).

A chi-square analysis was used on chicks' initial performance. As chicks' behaviour in the very first test trial is usually affected by the response to the novelty of the test situation (e.g. two screens are present in the arena), the first three trials were considered. A chick was regarded successful if it scored at least two correct trials within the first three trials, and this was the case for twenty-seven chicks, the remaining fourteen chicks were regarded as unsuccessful (they scored at least two mistakes in the first three trials). The difference (27 *Vs*. 14) being significant ($\chi^2_{(1)}$= 4.122; p=0.042).

The ANOVA on the response times showed no differences between the "correct" *Vs*. the "non-correct" trials ($F_{(1,38)}$= 0.327; p=0.570), nor a significant interaction (Response time x Experimental condition $F_{(2,38)}$= 0.499; p=0.611).

A statistically significant effect was found when analysing the average response times (considering together correct and non-correct responses) in the three experimental conditions ($F_{(2,38)}$= 18.239; p=0.0001; Colour: mean= 43.964; sem= 4.059; Size: mean= 65.239; sem= 5.121; Shape: mean= 30.865; sem= 3.974. See Fig. 13). Post-hoc using Fisher's LSD revealed that each condition differed from the other two (Colour *Vs.* Shape, p=0.027;

Shape *Vs.* Size, $p<0.0001$; Size *Vs.* Colour, $p=0.0014$). Chicks were faster when the two objects differed in shape, and slower when objects differed in size. Difference in response times are coherent with results obtained on other species. In fact, similarly to chicks, humans showed that differences in shape are the most salient feature (Leslie et al. 1998) compared to the other properties, while, for monkeys colour would be the most salient feature (Santos et al. 2002). Moreover, infants from 4.5 months use shape or size features for establishing that two objects are involved in an event but it is not until 7.5 months that they use pattern information and only at 11.5 months successfully use colour information for object individuation (Wilcox 1999). The critical age decreases at 4.5 months for pattern and 7.5 months for colour if infants are previously exposed to events in which the pattern or the colour of an object is experienced as predictive of its function; Wilcox & Chapa 2004).

Overall, the results of the first experiment show that chicks spontaneously individuate the larger number of objects making use of property information (Colour, Size or Shape).

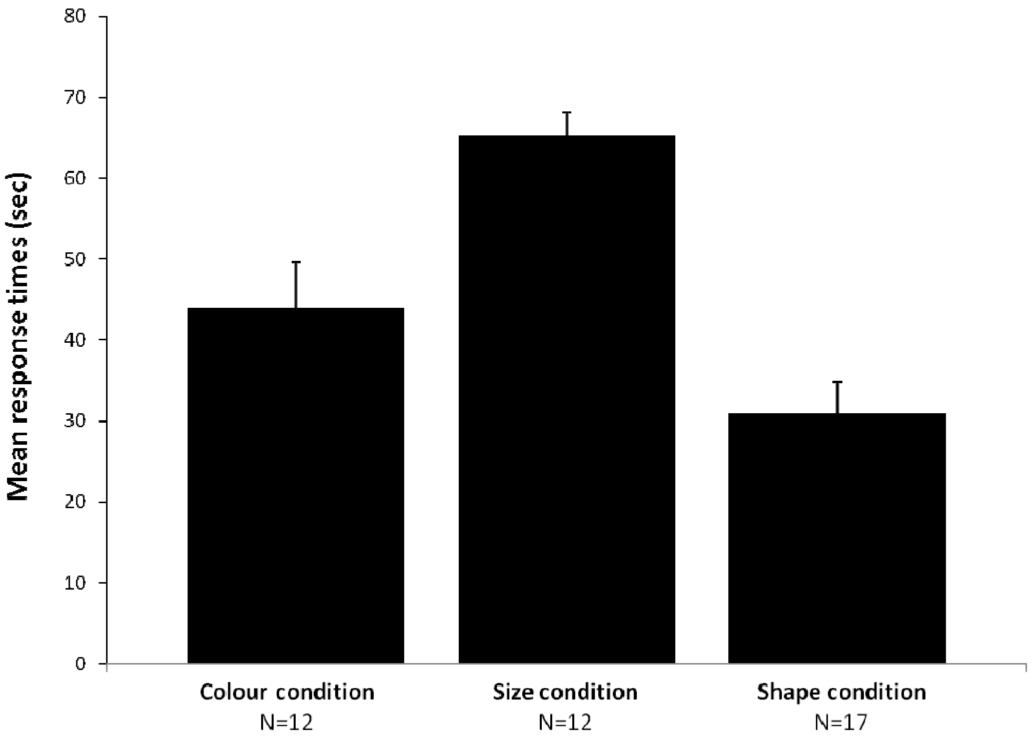


Fig. 13. Response times (group means with SEM are shown) for the three experimental conditions of Experiment. 1. All conditions differed from one another (see the text for more details).

EXPERIMENT 2

Experiment 1 supports the idea that chicks can individuate objects using property information provided by Colour, Shape or Size, in fact, in all cases, chicks chose the screen hiding two different objects. A possible objection to this conclusion would be that chicks chose two different objects because they may simply prefer a more varied group of events (two different stimuli could be preferred to two identical stimuli). To test this possibility a new group of chicks were presented with two identical *Vs.* two different objects, both objects in a same pair being simultaneously visible. In fact from previous literature there is clear (though indirect) evidence that chicks are able to individuate identical objects as separate if these are seen simultaneously (Rugani et al., 2009; 2010).

If chicks' responses favoured the higher number of objects, no screen would be preferred, as both screens concealed two objects. If, on the other hand, chicks were attracted by a more varied set of objects, then they would have preferred to search the screen hiding two different objects.

Being there in the previous experiment no difference due to objects features, the Colour condition was arbitrarily used in Experiment 2.

MATERIALS and METHODS

SUBJECTS, STIMULI AND PROCEDURE

A new group of 12 female chicks were tested. Imprinting and testing stimuli (see Fig. 11a), apparatus and general training procedures were identical for all chicks and identical to those used in the Colour condition of Experiment 1.

The Simultaneous Procedure test was used. Two events were sequentially presented, each event consisted in the simultaneous presentation of two objects: either two identical squares (e.g. yellow + yellow) or two squares of different colour (i.e. yellow + green).

RESULTS AND DISCUSSION

The mean percentage of correct responses did not differ from chance level (t(11)= -0.232, p=0.820; mean= 49.166; sem= 3.579. See Fig. 14 – Exp. 2). No statistically significant differences were there between response times for "correct" *Vs.* "non-correct" trials (paired t-test: t(11)= -1.013, p=0.332; "correct": mean= 32.968; sem= 3.781; "non-correct": mean=

34.820; sem= 4.616).

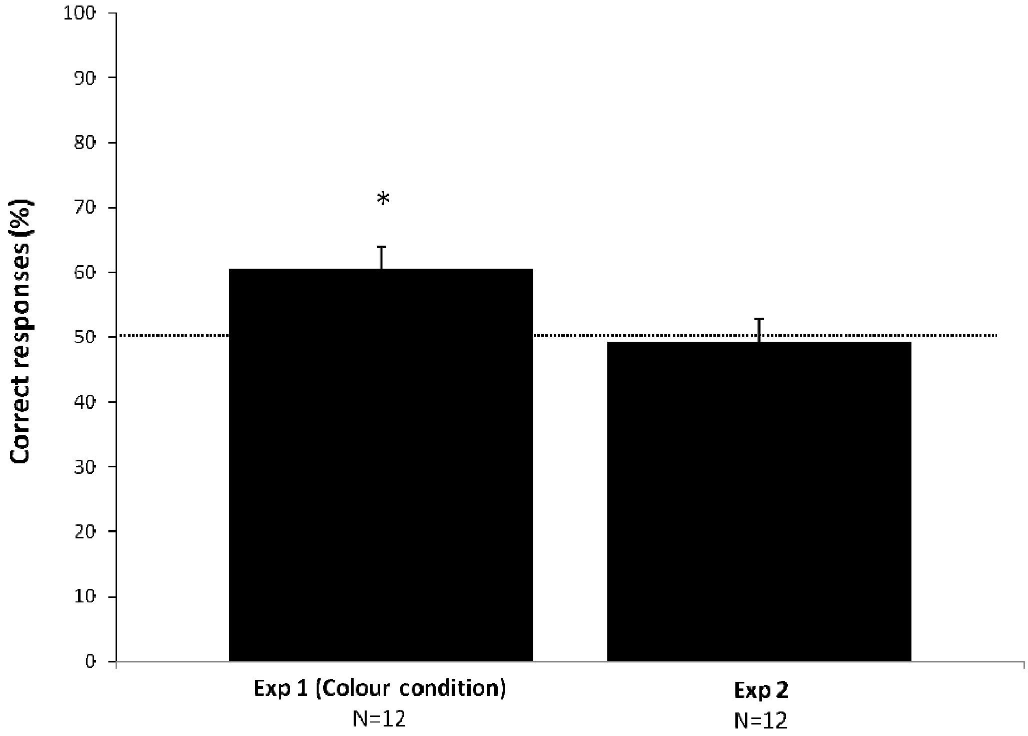


Fig. 14. Percentage of correct responses (i.e. choice for the screen hiding two different stimuli) scored in Experiment 1 for ‘Colour condition’ and Experiment 2 (group means with SEM are shown). Exp. 1: 2 *Vs*. 2 events comparison with sequential presentation of stimuli: one stimulus presented twice *Vs*. two different stimuli presented once each which differed for colour. Exp. 2: 2 identical objects *Vs*. 2 objects different for colour simultaneously presented.
The dotted line (y=50) represents chance level. * = p<0.001.

When two events are compared, each comprising a group of two objects presented simultaneously, the chicks did not show any preference, even though one group was made of two identical stimuli and the other was made of two stimuli differing for their colour. The lack of choice in this experiment exclude that chicks’ performance in the previous experiment was due to a general preference for the most varied set of stimuli. Those results might support the hypothesis that chicks' choice in Experiment 1 would be based on the use of property information for object individuation.

EXPERIMENT 3

In the third experiment, chicks' ability to use spatiotemporal information was assessed. Chicks were presented with a 2 *Vs.* 3 objects comparison in which each set of objects was presented simultaneously. Two different objects were compared with three identical objects for providing further support to the findings of Experiment 1 and 2, to rule out the possibility that chicks' choice depended on attraction for the more varied group of stimuli.

If chicks do use spatiotemporal information, they would approach the screen hiding the larger number of objects.

MATERIALS and METHODS

SUBJECTS, STIMULI AND PROCEDURE

A new group of 12 female chicks were tested. Rearing conditions were identical to those described in the general procedure.

Imprinting and testing stimuli, apparatus and general training procedures were identical for all chicks and identical to those used in Experiment 2. Test procedure was also similar to the Simultaneous procedure test, but three identical squares (e.g. yellow + yellow + yellow) were simultaneously presented and confronted with another event featuring the simultaneous presentation of two stimuli of different colour (i.e. yellow + green). The whole procedure took approximately 20 seconds, and after a delay of five seconds, the chick was released within the arena.

Stimuli used and their presentation were balanced at test. Correct choices (i.e., detour of the screen hiding three identical objects) were computed as percentages.

RESULTS AND DISCUSSION

Chicks preferred the screen hiding three identical objects (t(11)= 4.304; p=0.001; mean= 63.333; sem= 3.097. See Fig. 15). No difference was found between the response times for the "correct" *Vs.* the "non-correct" trials (t(11)= -0.334, p= 0.744; "correct": mean= 31.186; sem= 3.946; "non-correct": mean= 33.183; sem= 5.207).

As regards chicks' initial performance, seven chicks scored at least two correct trials within the first three trials, while five chicks scored at least two mistaken trials in the first three trials, the difference (7 *Vs*. 5) being non significant ($\chi^2_{(1)}$=0.333; p= 0.563).

Results suggested that chicks are able to use spatiotemporal information for establishing the numerical identity of objects presented in a visual scene, even though they do not perform above chance in the very first trials. It could be that comparison between two different and three identical stimuli, even though simultaneously, is more difficult. In fact, chicks during imprinting become familiar to an exact number of objects of different colours, namely three yellow and three green objects. This may make both sets, i.e. three identical or two different stimuli, actual subset of the imprinting stimuli rendering the choice more difficult.

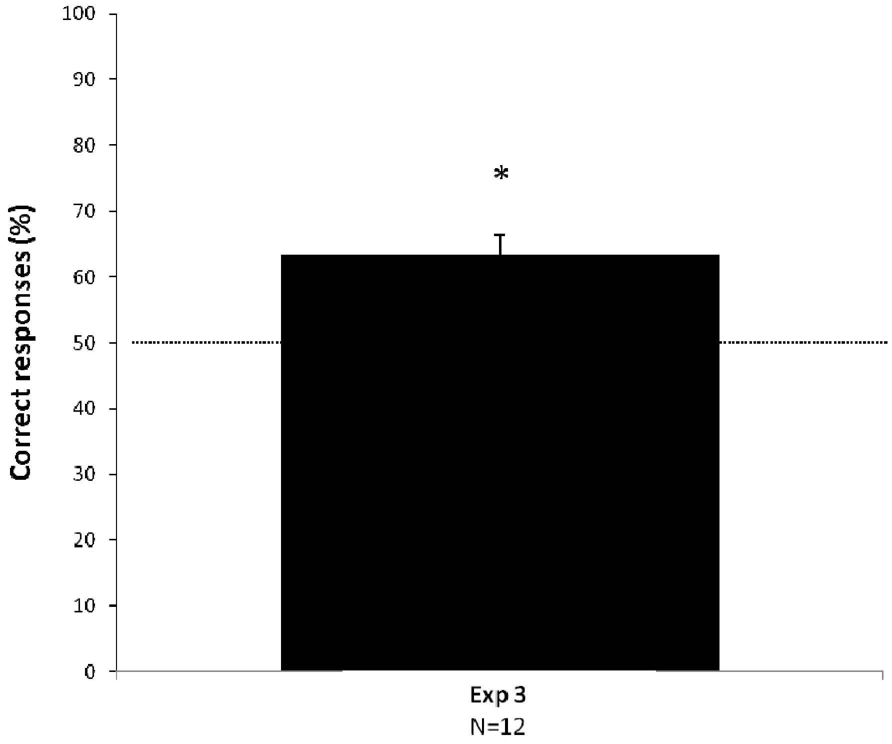


Fig. 15. Percentage of correct responses (i.e. choice for the screen hiding three identical stimuli) scored in Experiment 3 (group means with SEM are shown). 3 identical *Vs*. 2 different objects simultaneously presented.
The dotted line (y=50) represents chance level. * = p<0.001.

EXPERIMENT 4

From the previous experiment it appears that chicks are able to correctly identify up to three objects if spatiotemporal cues are available. A fourth experiment was carried out to assess whether, in the absence of such cues, increasing the number of events taking place would affect the use of property information. Chicks were required to choose between a larger set of identical objects contrasted with a smaller set of different objects; objects of both sets were presented in spatiotemporal discontinuity. The procedure was identical to that employed in the first experiment, except for the fact that one group of events was composed of a higher number (i.e., three) of events (i.e., one stimulus sequentially presented for three times) and was compared to a group of two events comprising two different stimuli, each sequentially presented once only.

Would the chick regard three presentations of the same object as “more” than two presentations each involving a different object?

MATERIALS and METHODS

SUBJECTS, STIMULI AND PROCEDURE

A new group of 12 female chicks were tested. Rearing conditions, apparatus, as well as imprinting and testing stimuli were identical to those already described in Experiment 3.

Testing procedure was also identical to that of Experiment 1 (Sequential presentation test) with the difference that one group of events was made of one stimulus sequentially presented for three times whereas the other group of events was made of two different stimuli sequentially presented once each. The whole procedure took approximately 45 seconds and the overall time of presentation of each group of events was comparable (to this purpose, for the group of three events the time of presentation of each object was reduced to six seconds). After a delay of five seconds, the chick was let free within the arena.

At test, the order of presentation of the groups of events as well as the screen of appearance and disappearance of the two sets were counterbalanced within each chick’s ten test trials, whereas the colour of the stimuli was randomised between subjects.

The percentage of correct choices (i.e. choice for the screen hiding two different stimuli) was computed considering the number of trials in which each chick chose the screen hiding the two different stimuli, and data were analyzed as for the previous experiments.

RESULTS AND DISCUSSION

The percentage of correct responses did not differ from chance level (t(11)= 0.000; p=1; mean= 50.000; sem= 3.692, see Fig. 17 – Exp. 4). Response times for the “correct” *Vs.* “non-correct” responses were different (t(11)= -2.594, p=0.024; “correct”: mean= 36.202; sem= 3.971; “non-correct”: mean= 42.450; sem= 3.936). Chicks were faster when choosing the correct screen as compared to the other screen.

Results showed that chicks’ did not prefer a larger number of presentations of one same stimulus (three events) to a smaller number of presentations of two different objects (two events).

EXPERIMENT 5

In the previous experiment, chicks did not show a preference between a group of two events made of two different stimuli and a group of three events made of one stimulus presented three times. Chicks’ lack of choice may indicate some limits in the process of object individuation. Alternatively, chicks may have identified three repeated presentations of a same stimulus as three different, though identical, objects. Due to rearing conditions, chicks were familiar with the fact that up to three distinct but identical objects can be actually encountered. Furthermore, both sets might be considered as correct because they had been exposed during imprinting familiarization to similar sets of objects (i.e. three yellow and three green objects). It seems that chicks compensate between the tendency to approach the larger number of presentations (i.e. one single object presented three times) and the smaller number of presentations but comprising two different objects. Differences in colour make more plausible that two different individuals are involved (i.e. two different objects presented once each).

Experiment 5 was devised to deal with this issue. In this experiment chicks experienced during rearing objects that were all different from one another: a set of five imprinting stimuli differing in their individual features was employed. Chicks were then tested in a 3 *Vs.* 2 events comparison, one stimulus presented three times *Vs.* two different stimuli presented once each.

MATERIALS and METHODS

SUBJECTS, STIMULI AND PROCEDURE

A new group of 13 female chicks were tested. Rearing conditions were identical to those previously described. Imprinting and test stimuli consisted in a group of five two-dimensional yellow squares (4 x 4 cm). Each stimulus differed from the others by the relative orientation of two black segments (each 2 cm long x 0.90 mm wide), printed on both sides of each square (Fig. 16). Previous studies demonstrated that chicks are sensitive to this kind of features, which would allow for individual object recognition (Vallortigara & Andrew, 1991; Vallortigara & Andrew, 1994).

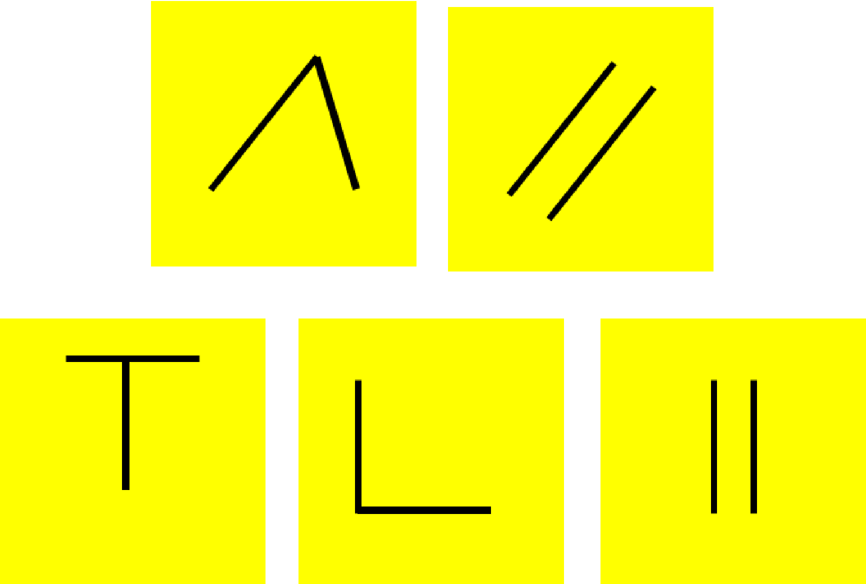


Fig. 16. The five different stimuli used for imprinting, training and test in Experiment 5.

The apparatus and the general training and testing procedures were identical to those of Experiment 4. All the stimuli employed in the test phase were different from each other and were also different from the single stimulus which was used during training (which stimulus was used at training was randomized across subjects).

The order of the events, as well as the screen (left–right) of appearance and disappearance of the two sets were counterbalanced within each chick’s ten test trials. Moreover, each stimulus was randomly assigned to either group of events trial by trial, in order to avoid effects due to learning.

The percentage of correct choices was computed considering as correct the trials in which the chick chose the screen hiding two different stimuli.

RESULTS AND DISCUSSION

Chicks preferred the screen hiding two different objects (t(12)= 5.333; p<0.001; mean= 62.307; sem= 2.307. See Fig. 17 – Exp. 5). No difference was found between the response times for the "correct" *Vs*. the "non-correct" trials (t(12)= -1.559, p=0.144; "correct": mean= 69.874; sem= 5.344; "non-correct": mean= 78.331; sem= 7.770).

As regards chicks' initial performance, ten chicks scored at least two correct trials within the first three trials, and three chicks scored at least two mistaken trials within of the first three trials. The difference (10 *Vs*. 3) being marginally not significant ($\chi^2_{(1)}$= 3.769; p=0.052).

Results support the hypothesis that chicks can use the property information provided by a peculiar feature of the stimuli for individuating the larger number of elements irrespective (and against) the number of events. Differently from the previous experiment, in which chicks failed, here chicks had become familiar with five unique individuals prior of test, making it unlikely that a repeated presentation of the same individual would be computed as two or even three distinct and identical individuals.

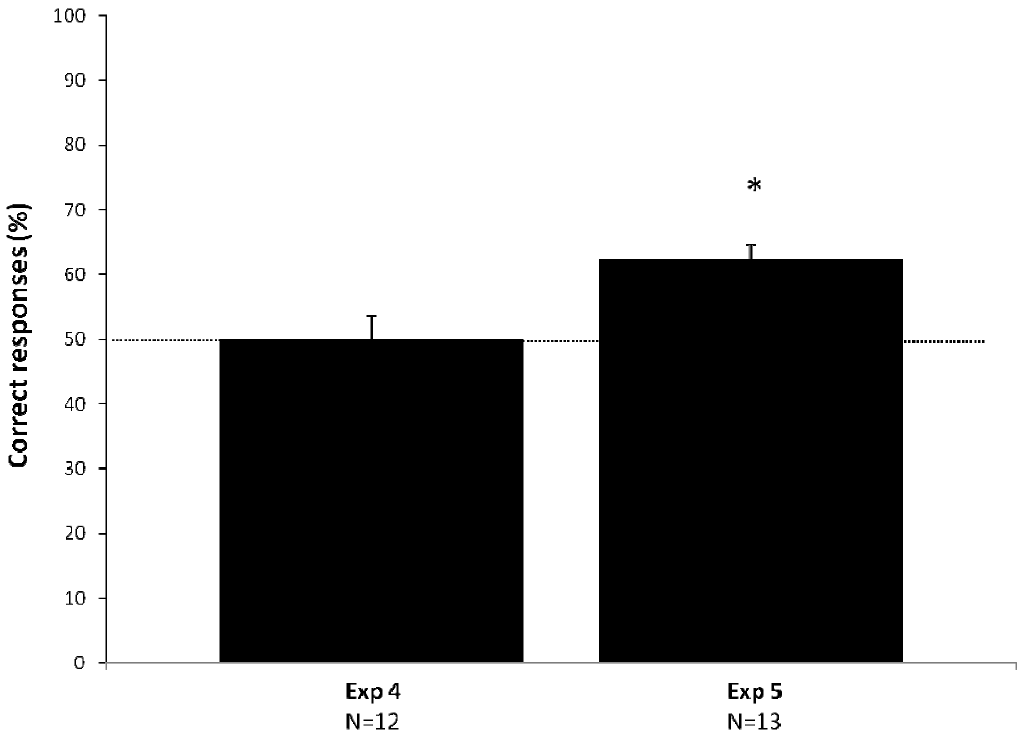


Fig. 17. Percentage of correct responses (i.e. choice for the screen hiding two different stimuli) scored in Experiment 4 and Experiment 5 (group means with SEM are shown). Exp. 4: 3 *Vs*. 2 events, with sequential presentation of stimuli; one stimulus presented three times compared to two different stimuli presented once each. Exp. 5: 3 *Vs*. 2 events comparison with stimuli each differing one from the other for individual features.
The dotted line (y=50) represents chance level. * = p<0.001.

EXPERIMENT 6

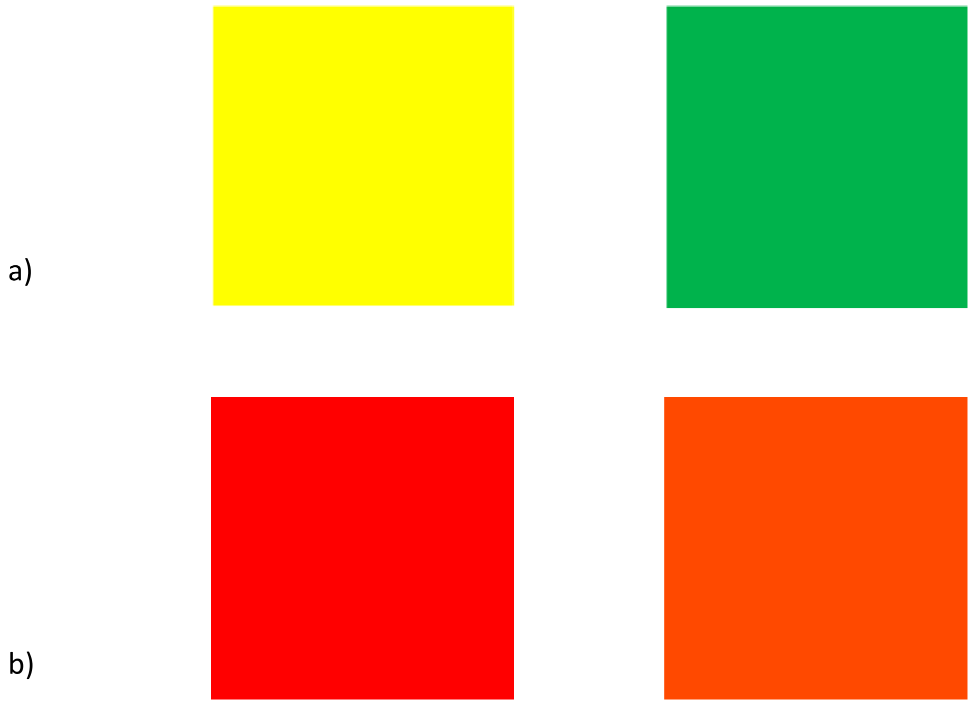
In the previous experiments chicks were proved able to use spatiotemporal as well as property information provided by colour, shape, size and individually distinct features for object individuation. Chicks were required to individuate objects to which they were exposed for the first two days of life; towards the process of filial imprinting chicks have developed social attachment to those objects. Through the process of filial imprinting, chicks learn the features of the object forming a recognition memory of that object. Even though they demonstrate to possess the ability to spontaneously use property information at three days of life, they do so after familiarization following exposition to the objects. The purpose of this experiment was to investigate whether chicks make use of property information also when provided by novel colours of familiar shaped objects for object individuation. Therefore, the aim is to determine whether previous, even though controlled, experience is needed for individuate objects. From an adaptive view, since chicks belong to a precocial species showing at hatching a behavior virtually identical to that of the adult animal (i.e. moving and feeding independently) it is plausible they would adapt soon to face objects of which they have never had experience in the natural environment. Changes in colour are treated by chicks as chances in the identity of the objects even though all the other features are held constant which makes new coloured objects particularly different individuals (Regolin et al., 2005).

Chicks were tested in a 2 *Vs.* 2 events comparison (i.e., one object presented twice *Vs*. two different objects each presented once). Therefore, if chicks use the new property information for individuating objects, they should approach the group of events featuring two different objects; if chicks do not use such information, they should behave at random, as they are faced with a choice between two sets comprising a same number of events.

MATERIALS and METHODS

SUBJECTS, STIMULI AND PROCEDURE

Subjects were a new group of 26 female chicks. Rearing conditions, apparatus and general training procedures were identical for all the chicks and identical to those previously described in the general procedure. For 14 subjects, imprinting stimuli were identical to those employed for the ‘Colour condition’ of Experiment 1: three green squares and three yellow squares (4 x 4 cm); at test they were presented with identical squares but coloured either in orange and red which they have not seen before (orange-red condition, see Fig. 18 a and b). For the other 12 chicks, imprinting stimuli were three green and three pink squares (4 x 4 cm) and test stimuli were yellow and orange squares (orange-yellow condition, see Fig. 18 c and d).



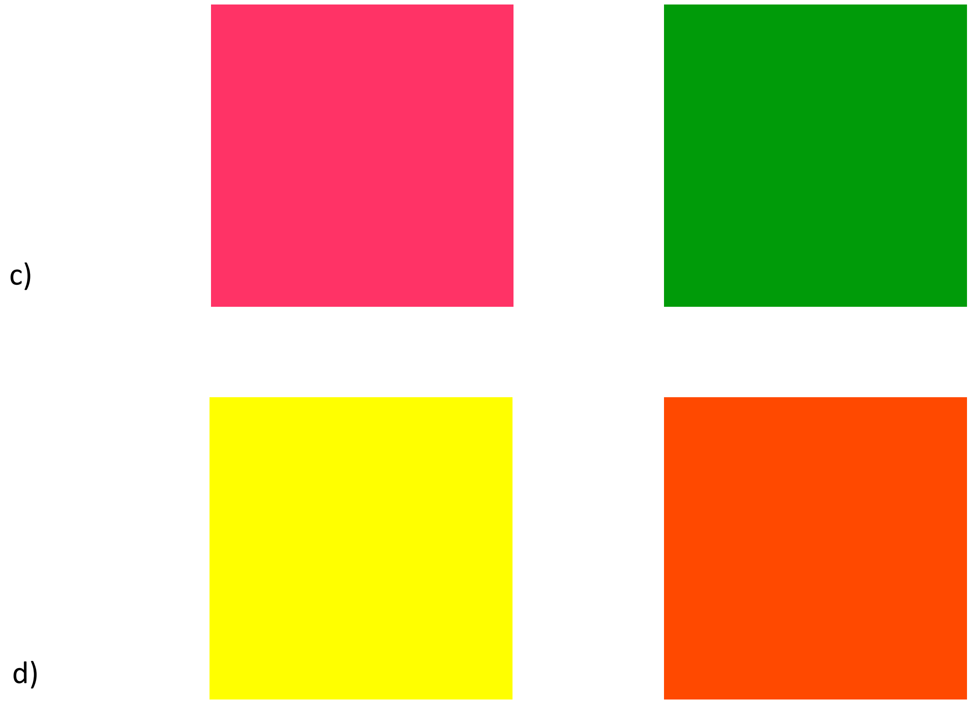


Fig. 18. The two types of objects used in Experiment 6 for imprinting training and test in each of the two experimental conditions. Orange-red condition: a) imprinting and training stimuli; b) test stimuli. Orange-yellow condition: c) imprinting and training stimuli; d) test stimuli.

Chicks underwent a Sequential Presentation test: one stimulus presented twice *Vs*. two different stimuli presented once each.

The number of trials in which each chick chose the screen hiding two stimuli differing for one property (which was regarded as the correct choice) was considered and percentages were computed as: (number of correct choices/10) x 100.

RESULTS AND DISCUSSION

No statistically significant difference in performance was found between the two conditions (two-sample-non paired t-test t(24)= -0.683, p=0.529; ‘orange-red condition’ mean= 57.142; sem= 4.964; ‘orange-yellow condition’ mean= 61.666; sem= 5.050). Data were therefore merged and the overall mean was compared with chance (50%) through a one sample t-test. Chicks preferentially approached the screen hiding two different objects (t(25)= 2.633; p=0.014; mean= 59.230; sem= 3.504. See Fig. 19). No difference was found between the response times for the “correct” *Vs*. the “non-correct” trials (t(25)= -0.560, p=0.580; “correct”: mean= 42.885; sem= 3.718; “non-correct”: mean= 45.943; sem= 5.710).

As regards chicks’ initial performance, 15 chicks scored at least two correct trials within the first three trials, and 11 chicks scored at least two mistaken trials within of the first three trials. The difference (15 *Vs*. 11) being not significant ($\chi^2_{(1)}$= 0.615; p=0.432).

Results demonstrated that chicks are able to use property information provided by novel colour for object individuation, choosing the screen hiding two different objects.

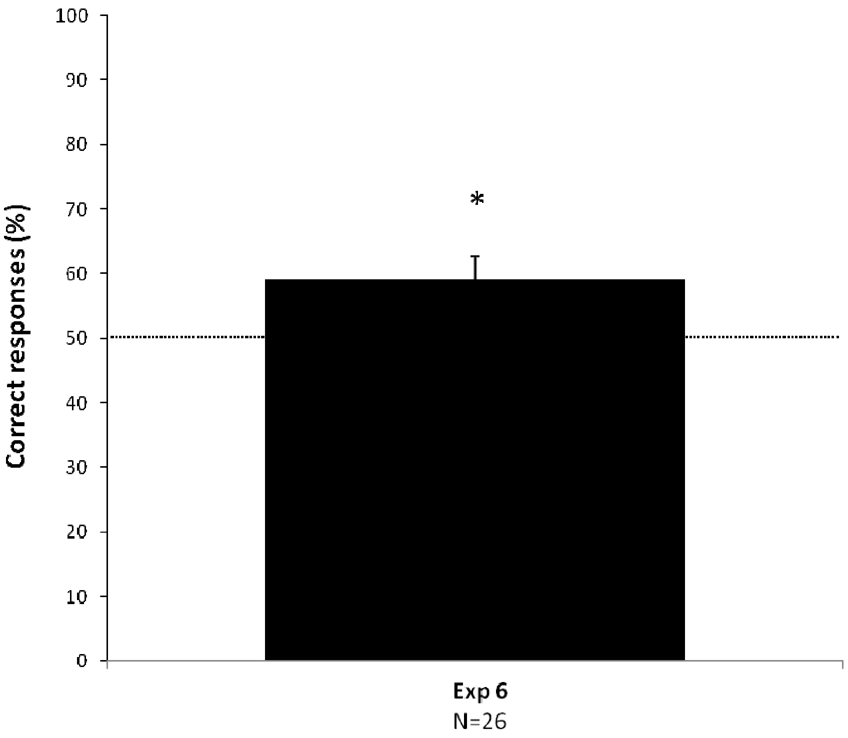


Fig. 19. Percentage of correct responses (i.e. choice for the screen hiding two different stimuli) scored in Experiment 6 (group means with SEM are shown). 2 *Vs*. 2 events comparison with sequential presentation of novel stimuli (as compared to the imprinting stimuli): one stimulus presented twice *Vs*. two different stimuli presented once each.
The dotted line (y=50) represents chance level. * = p=0.014.

PROPERTY/KIND INFORMATION

The aim of this second series of experiments was to investigate the phylogenetic development of the ability to use property/kind information for object individuation. This research represents the first attempt of studying the current topic in a bird species, with the purpose to draw a link between abilities possessed by different classes of animals, determining whether this ability evolved early in phylogenesis.

As already stressed, domestic chicks are precocial birds, able to follow the mother hen and the siblings and to feed independently soon after birth. For this reason I believed it plausible that they precociously exhibit some competences concerning the categories of objects most crucial to their survival. Rudimental representations of object categories may in fact prove useful for an animal dealing with its own environment and may therefore result in an increased fitness.

EXPERIMENT 7

Feeding and social behaviour certainly constitute the two main domains in the chick's early life. Thus, food and social stimuli were used in a paradigm analogous to that previously used for the investigation of property information in this same species.

Both categories are crucial to the newborn's survival, though they imply very different behavioural responses, and plausibly different levels of motivation are associated with either domain under differential environmental conditions. The first experiment investigates under which conditions chicks would be equally motivated to respond to food or to social stimuli. As previous studies demonstrated that chicks tend to approach the larger group of objects (Rugani et al., 2009; 2010), chicks were tested with two sets of identical size. Chicks were presented with a 2 *Vs.* 2 stimuli comparison (two social stimuli *Vs.* two food stimuli, both elements in a set being simultaneously presented). If chicks should choose any of the two groups of stimuli, their behavior would likely indicate a preference for that category of objects. No preference would be displayed under conditions in which no bias is present towards one category of object.

Lack of interest for the two attractors could also explain lack of preferential choice, and therefore should be ruled out beforehand. To this purpose, prior of the actual test, subjects underwent two tests designed to probe motivation to, respectively, social stimuli (Imprinting Motivational test) and food stimuli (Food Motivational test). Only those chicks who promptly responded to both stimulus categories moved on to the actual test.

This seventh experiment aimed at establishing the proper conditions in which chicks were equally motivated to both social and food stimuli.

MATERIALS and METHODS

SUBJECTS STIMULI AND REARING CONDITIONS

Subjects were a new group of 7 female chicks. Rearing conditions and apparatus were identical for all the chicks and identical to those previously described in the general procedure. Imprinting stimuli were five identical rounded objects made of yellow plastic. Each object was a ‘Kinder surprise’ capsule (Ferrero S.P.A. Alba, Cuneo, Italy) measuring 4 x 3 x 3 cm (which we will hereafter refer to as a “ball”). Chicks were fed with mealworms (*Tenebrio molitor* larvae) which will later constitute the food stimuli (see Fig. 20 for an example of the stimuli). In the morning of day 1 (once housed in the home cage) one mealworm was gently offered to the bird (by picking it up with tweezers and keeping it in front of the chick's beak to prompt pecking). Three hours later, a second mealworm was placed on the floor of the home cage, in order to let chicks catching and eating it. Other two mealworms were given on day 2, one in the morning and one in the afternoon. Only chicks that ate all mealworms entered the training phase. Chicks were kept in the standard rearing conditions described above for three days: from the morning (11.00) of the first day (i.e. Monday, the day of their arrival, which was considered as day 1) to the third day (Wednesday, day 3). In the afternoon (14.00) of day 3, they underwent the training. Approximately one hour later, they took part in the motivational tests and approximately one hour after such motivational tests they underwent the proper testing session.

Fig. 20. Example of the social stimuli (on the left) and food stimuli (on the right) employed for investigating property/kind information.

PROCEDURE

Training

In the afternoon of day 3, chicks underwent a preliminary training session identical to the general training procedure with the only difference that the stimuli employed were both social as well as food stimuli. Firstly, chicks learned to rejoin for two consecutive times the imprinting stimulus hidden behind the screen.

Then, the food stimulus (one mealworm, suspended by a fine thread) was employed using the same procedure. The training ended when the chick had rejoined the food stimulus for two consecutive times. On average, to complete the training a chick required approximately 15/20 minutes.

Motivational tests

Before the test and about one hour after the training, each chick (once successfully completed the training phase) underwent two motivational tests (i.e., Imprinting and Food Motivational test). The aim of such tests was to make sure that chicks would adequately respond to both attractors in a situation similar to that later faced at test (response times scored in each motivational test for each experiment are reported in the Appendix).

Imprinting Motivational Test: the same training procedure described above was used, employing one imprinting stimulus. The chick was required to rejoin its imprinting stimulus once it had disappeared behind the screen in 10 consecutive trials and the response time (seconds) was measured. If during one trial a chick did not approach the screen within three minutes, the trial was considered null and void and it was repeated immediately afterwards. After three consecutive null trials, the chick was discarded from the experiment (this occurred for about 5% of chicks, not included in the final sample).

Food Motivational Test: eight pieces of mealworm were placed on a white cardboard (18 x 30 cm) spaced out about 4 cm from one another. The cardboard was placed in the middle of the experimental arena and the time (seconds) needed by the chick for eating all pieces of mealworm was scored. If the chick did not eat all eight pieces of mealworms within five minutes it was discarded from the experiment (this occurred in about 20% of chicks, not included in the final sample).

The order of presentation of the motivational tests was randomized across subjects.

Testing

Only about 50% of the birds completed the training phase (about 25% of subjects did not reach the criteria and were not included in the final sample) and both motivational tests (about 25% of subjects did not reach the criteria and were not included in the final sample), these birds underwent the testing phase.

The testing phase took place one hour after the end of the motivational tests and the Simultaneous Presentation test was performed. The chick was shown with two separate events, each of them consisted in the simultaneous presentation of two attractors (two imprinting or two food stimuli); the two events were shown consecutively, i.e., the second event took place just after the end of the first one, the order of presentation being randomized. Before the beginning of each trial, all stimuli were positioned behind the screens. Two extra pieces of mealworm (which would constitute the reward) were also placed behind the screen hiding the food stimuli.

At the end of each trial, depending of which screen was chosen chicks were allowed to spend one-two seconds together with their ‘social companions’ or to eat the pieces of mealworm present behind the screen.

The number of trials in which each chick chose the screen hiding the food stimuli and the social stimuli was considered and percentages were computed as in the previous experiments (number of correct choices/10) x 100.

RESULTS AND DISCUSSION

Chicks preferred the screen hiding two food stimuli (t(6)= 13.554; p<0.0001; mean= 77.142; sem= 5.654. See Fig. 21 – Exp. 7). No difference was found between the response times for the "food stimuli" *Vs*. the "social stimuli" trials (t(6)= -0.838, p=0.434; "food stimuli": mean= 8.195; sem= 0.910; "social stimuli": mean= 11.052; sem= 3.561). Results demonstrated that chicks, in this rearing and testing conditions, preferred the screen hiding two food stimuli. Motivation for the food attractor in the afternoon of the third day of life seemed to be higher than motivation for the social attractor. It could be that having experienced a total amount of four mealworms in two days, motivation for these stimuli would be enhanced compared to social stimuli. Moreover, the colour of the balls used as stimuli and the day of testing could not be enough attractive for triggering motivation towards the social object as motivation for food.

EXPERIMENT 8

In Experiment 7, chicks were reared with yellow balls and fed four mealworms overall during the two days of rearing. In a Simultaneous presentation of two food stimuli *Vs.* two imprinting stimuli, chicks preferred the screen hiding food stimuli. In order to find conditions in which chicks are comparable motivated to those categories, in this experiment several manipulations had been provided. First, the attractiveness of the imprinting stimuli was increased employing balls of the same shape but of more attractive colours for chicks, such as red (Vallortigara, Regolin, & Pagni, 1999). Second, the number of mealworms was halved: chicks were fed with two mealworms. Third, the test was performed on the second day of life (so, it was one day in advance) because chicks at hatching are endowed with yolk sac reserves in the abdomen that provides nourishment for the first 72 hours of life. If chicks are presented with food attractors when the natural food store is still available, they might be less attracted by food stimuli. Moreover, the attractiveness for social stimuli might increase because of chicks being tested during the crucial hours for triggering social attachment towards the process of filial imprinting.

MATERIALS and METHODS

SUBJECTS STIMULI AND REARING CONDITIONS

Subjects were 12 female chicks reared as described in the general procedure. Apparatus, training, motivational test and testing were identical to those described in the previous experiment. Imprinting stimuli were identical to those employed in Experiment 7, but the five plastic balls were red in colour. Chicks were fed with two mealworms (*Tenebrio molitor* larvae) which will later constitute the food stimuli: in the morning of day 1 (once housed in the home cage) one mealworm was gently offered to the bird (by picking it up with tweezers and keeping it in front of the chick’s beak to prompt pecking). Three hours later, a second mealworm was placed on the floor of the home cage, in order to let chicks catch and eat it. Only chicks that ate both mealworms entered the training phase.

Chicks were kept in the standard rearing conditions described above for two days: from the morning (11.00) of the first day (i.e. Monday, the day of their arrival, which was considered as day 1) to the day after (Tuesday, day 2). In the afternoon (14.00) of day 2, they underwent the training. Approximately one hour later, they took part in the motivational tests and approximately one hour after such motivational tests they underwent the actual testing session.

PROCEDURE

Training, motivational tests and actual test were identical to those described for the previous experiment. The stimuli were red balls and mealworms.

RESULTS AND DISCUSSION

Chicks' performance at test did not differ from chance level (50%) (one-sample t-test t(11)= 1.448; p=0.175; "food stimuli"= 54.166; sem= 2.875. See Fig. 21 – Exp. 8) and no differences in reaction time between "food stimuli" and "social stimuli" trials were found (t(11)= -1.437, p=0.178; "food stimuli": mean= 9.719 sec; sem= 2.677; "social stimuli": mean= 20.220 sec; sem= 8.393).

Chicks did not preferentially choose between a group of two social stimuli and a group of two food stimuli, each set being simultaneously presented, even though they had proved to be motivated to respond to both type of stimuli beforehand. Lack of choice was considered to indicate same motivation towards food or social target.

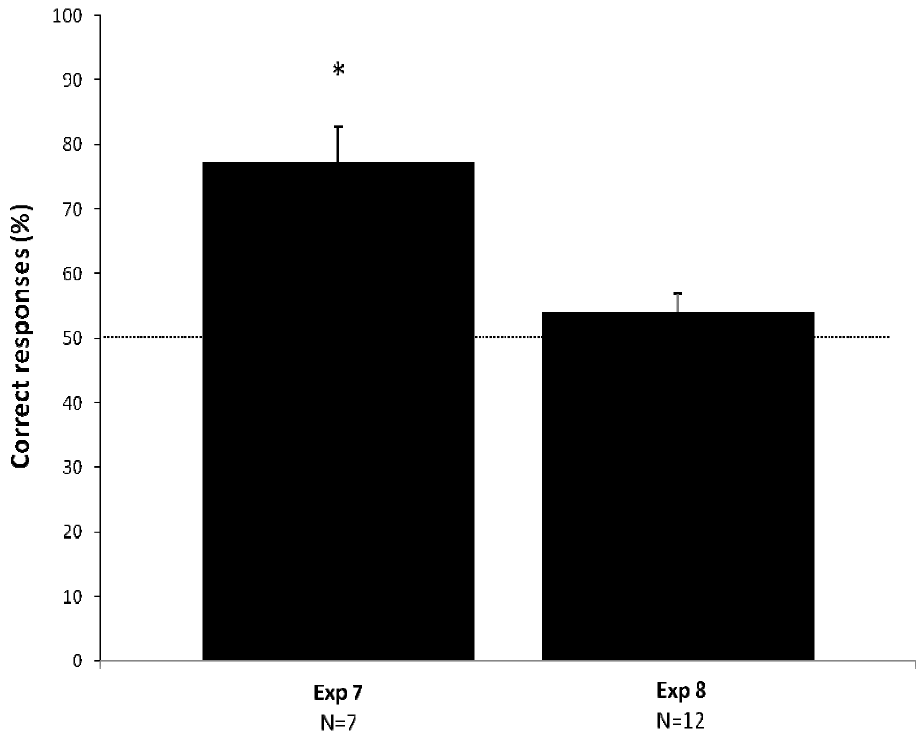


Fig. 21. Percentage of correct responses scored in Experiment 7 and Experiment 8 (group means with SEM are shown). Exp. 7: simultaneous presentation of two yellow imprinting balls *Vs*. two food stimuli, test carried out on the third day of life. Exp. 8: simultaneous presentation of two red imprinting balls *Vs*. two food stimuli, test carried out on the second day of life.
The dotted line (y=50) represents chance level. * = p<0.001.

EXPERIMENT 9

Under the conditions of the previous experiment, chicks appeared to be equally motivated to social and food attractors. Employing same rearing conditions as well as same stimuli of Experiment 8, in this experiment we investigated whether chicks make use of property/kind information provided by imprinting and food items for object individuation. Chicks were tested in a comparison between one stimulus presented twice *Vs.* two different stimuli each presented once. Previous studies demonstrated that chicks, in a discrimination task, tend to approach the larger group of either social or food stimuli (Rugani et al., 2009; Rugani et al., 2010; Regolin et al., 2010).

Here, chicks were presented with an identical number of events (i.e. 2 events) taking place by each screen, though in one case only one object was involved, whereas in the other case two objects were involved. Two objects differing for property/kind are for sure distinct, while the repeated presentation of one same object could either refer to two distinct, though identical, objects or to one same object presented twice.

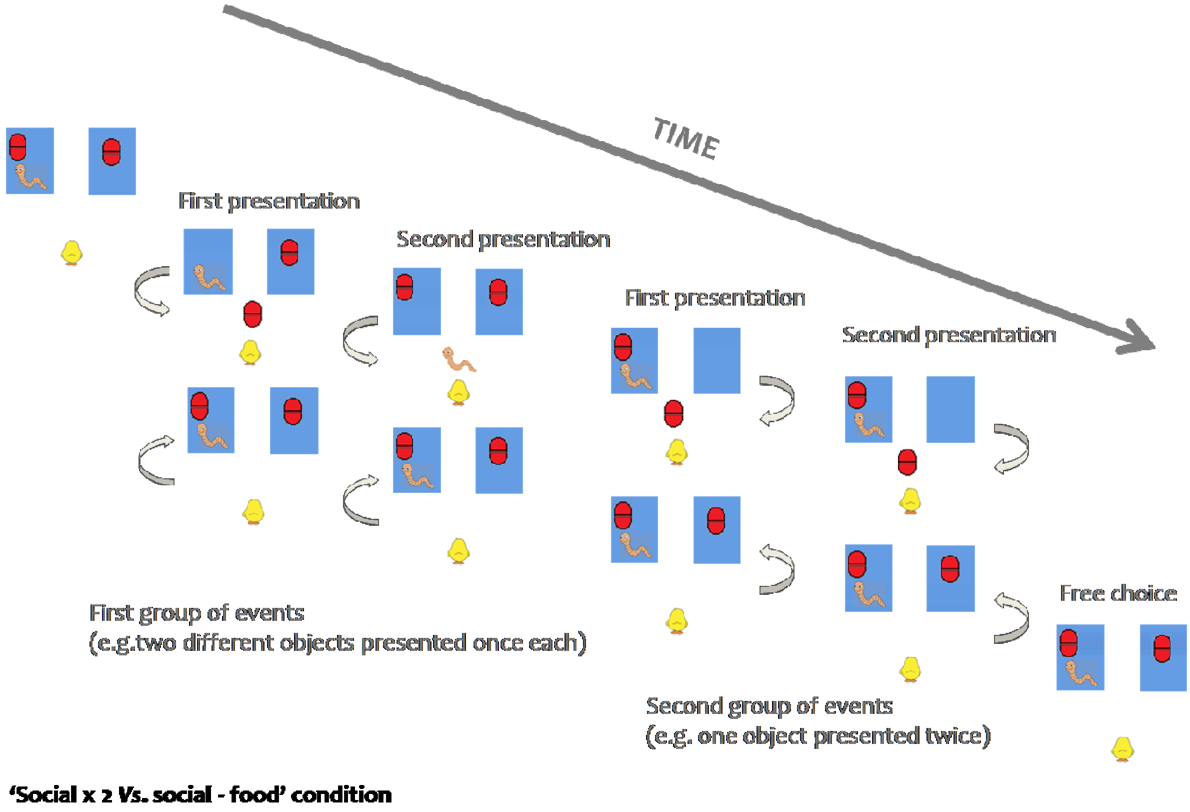
The assumption is that if chicks use property/kind information they should approach the screen hiding two different objects, because the difference in property/kind would suggest it more likely the presence of two distinct objects (i.e., of a larger group). Otherwise chicks should not manifest a preferential choice for the set comprising two different objects (given that they do not prefer either category to start with).

MATERIALS and METHODS

SUBJECTS, STIMULI AND PROCEDURE

Subjects were a new group of 25 female chicks. Imprinting and testing stimuli, apparatus, general training and motivational tests were identical to those described in Experiment 8. A Sequential presentation test was performed presenting two different stimuli (i.e. an imprinting and a food stimulus) *Vs*. one stimulus presented twice (i.e. either an imprinting ball or a mealworm). Twelve female chicks took part in the 'social x 2 *Vs.* social - food' condition (i.e. one imprinting stimulus presented twice *Vs.* the two different stimuli presented once each) and thirteen female chicks took part in the 'food x 2 *Vs.* food - social' condition (i.e. one mealworm presented twice *Vs.* the two different stimuli presented once each). See Fig. 22 for an example of the procedure employed.

The number of trials in which each chick chose the screen hiding two different stimuli (which was regarded as the correct choice) was considered and percentages were computed as: (number of correct choices/10) x 100. A two-sample-non-paired t-test compared percentage of correct choices scored in the 'social x 2 *Vs.* social - food' condition and 'food x 2 *Vs*. food - social' condition and one-sample two-tailed t-test (to assess significant departures from chance level, i.e. 50%) were used. A two-sample-paired t-test was computed for mean reaction time scored for "correct" and "non-correct" choices.



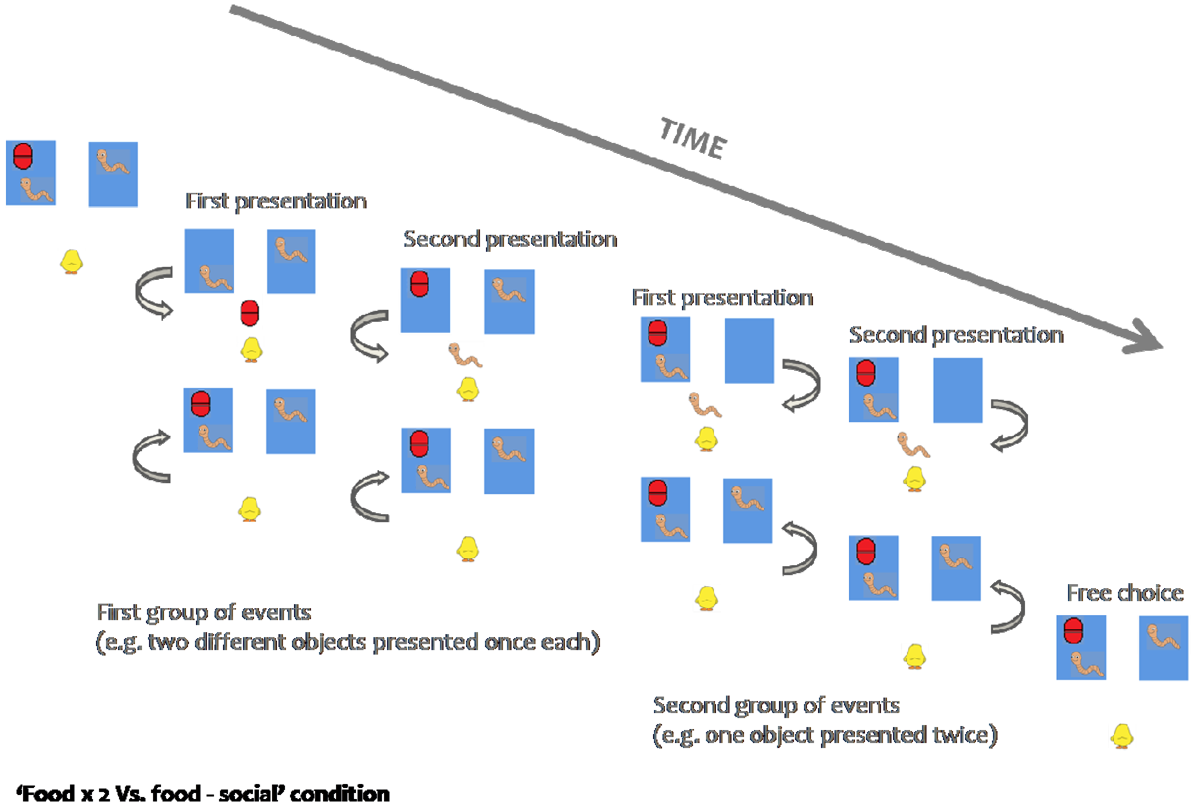


Fig. 22. Procedure of Experiment 9. Example of presentation of the stimuli for the 'social x 2 *Vs*. social - food' condition and the 'food x 2 *Vs*. food - social' condition. To better illustrate the procedure, in the picture, the objects are visible while placed behind the screens as if these were transparent. A first group of events involved a single presentation of each of two different stimuli (a ball and a mealworm), presented one after the other. The second group of events employed the consecutive presentation of one same stimulus twice (a mealworm for 'food x 2 *Vs*. food -social' condition and a ball for 'social x 2 *Vs*. social - food' condition).

RESULTS AND DISCUSSION

No statistically significant difference in performance was found between the two conditions (two-sample-non paired t-test t(23)= -1.695, p=0.103; 'food x 2 *Vs*. food - social' mean= 56.153; sem= 2.664; 'social x 2 *Vs*. social - food' mean= 63.333; sem= 3.333). Data were therefore merged and the overall mean was compared with chance (50%) through a one sample t-test. Chicks preferentially approached the screen hiding two different objects (t(24)= 4.369; p=0.0002; mean= 59.600; sem= 2.196. See Fig. 23 – Exp.9). A chi-square analysis was used on chicks' initial performance for assessing whether chicks' choices were due to learning across trials. As chicks' behaviour in the very first test trial is usually affected by the response to the novelty of the test situation (e.g. two screens are for the very first time experienced within the arena), the first three trials were considered. A chick was regarded successful if it scored at least two correct trials within the first three trials, and this was the case for twenty chicks, the remaining five chicks were regarded as unsuccessful (they scored at least two mistakes in the first three trials). The difference (20 out of 25) being significant ($\chi^2_{(1)}$= 9; p=0.002). A two-sample-paired t-test on the response times showed no differences between the "correct" *Vs*. the "non-correct" trials (t(24)= 0.207; p= 0.837; "correct": mean= 12.680; sem= 2.744; "non-correct": mean= 12.222; sem= 1.922).

Chicks tested in a 2 *Vs*. 2 events comparison with one stimulus presented twice *Vs*. two different stimuli each presented once choose the group of events featuring two different stimuli, suggesting that they made use of property/kind information provided by imprinting and food items for individuating the larger group of objects. Alternatively, chicks may choose the group comprising an imprinting and a food objects because they simply prefer a more varied group of stimuli. Experiment 10 was designed to test this possibility.

EXPERIMENT 10

Experiment 9 supports the hypothesis that chicks can individuate objects using property/kind information featuring social and food items. A possible objection would be that chicks' choice of two different objects is due to a preference for the more varied group of events (two different stimuli are preferred to two identical stimuli). In order to test this possibility a new group of chicks were presented with two identical *Vs.* two different stimuli. Both stimuli in a same pair being simultaneously visible, and therefore immediately individuated by the subject.

If chicks' responses favoured the higher number of stimuli, no screen would be preferred, as both screens concealed two objects. If, on the other hand, chicks were attracted by a more varied set of objects, then they would prefer to search the screen hiding two different objects.

MATERIALS and METHODS

SUBJECTS, STIMULI AND PROCEDURE

A new group of 28 female chicks were tested. Half of them took part in the 'social+social *Vs*. social+food' condition (i.e. two red balls *Vs*. one red ball and a mealworm) whereas the other half took part in the 'food+food *Vs*. food+social' condition (i.e. two mealworms *Vs.* a mealworm and one red ball). Imprinting and testing stimuli, apparatus, general training and motivational tests procedures were identical to those described in Experiment 8.

At test two events were sequentially presented, each event consisted in the simultaneous presentation of two stimuli: either two identical imprinting stimuli (i.e. two red balls) or two food stimuli (i.e. two mealworms) according to the experimental conditions (‘social+social *Vs*. social+food’ and ‘food+food *Vs*. food+social’) or two different stimuli (i.e. one red ball + one mealworm). In each event the two stimuli were made simultaneously appearing from one screen, coming in front of the chick confined in the holding box and then made to slowly disappear behind the same screen. The whole procedure took approximately 20 seconds. After a delay of five seconds, the chick was let free within the arena.

The order of the events as well as which screen concealed which event were counterbalanced within each chick’s ten testing trials. The detour of the screen hiding two different stimuli was arbitrarily chosen as correct choice, and percentages were computed, as described for the previous experiment.

RESULTS AND DISCUSSION

There was no statistical difference between performance in the two experimental conditions as revealed by the two-sample-non paired t-test (t(26)= -1.067, p=0.296; ‘social+social *Vs*. social+food’ mean= 47.142; sem= 5.389; ‘food+food *Vs*. food+social’ mean= 53.571; sem= 2.694). Data were therefore merged and the overall mean was compared with chance (50%) through a one sample t-test. The mean percentage of correct choice did not differ from chance level (t(27)= 0.1182; p=0.9067; mean= 50.357; sem= 3.020. See Fig. 23 – Exp. 10). A two-sample-paired t-test on the response times showed no differences between “correct” *Vs*. “non-correct” trials (t(27)= -1.317; p=0.198; “correct”: mean= 11.347; sem= 2.107; “non-correct”: mean= 15.284; sem= 4.350).

Chicks did not prefer two different objects to two identical objects, each pair simultaneously presented. This lack of choice excludes the possibility that in the previous experiment chicks chose the group of two different stimuli (sequentially presented) because of a preference for the more varied set of objects, therefore supporting the finding of Experiment 9, that chicks use property/kind information provided by social and food stimuli for object individuation.

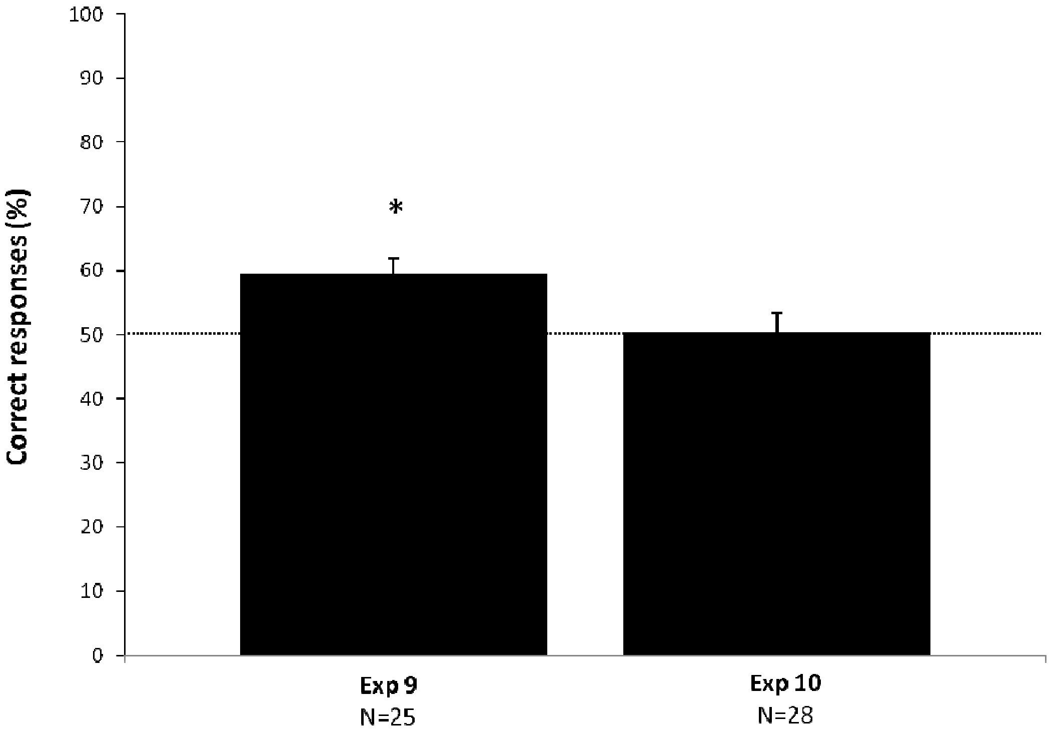


Fig. 23. Percentage of correct responses scored in Experiment 9 and Experiment 10 (group means with SEM are shown). Exp. 9: One stimulus presented twice *Vs*. two different stimuli presented once each (2 *Vs*. 2 events), the stimuli were presented sequentially. Exp. 10: Two identical stimuli *Vs*. two different stimuli, simultaneously presented.
The dotted line (y=50) represents chance level. * = $p<0.001$.

GENERAL DISCUSSION AND CONCLUSIONS

The aim of the present research was to investigate object individuation ability for the first time in a bird species and in very young individuals. In particular, the use of property information, spatiotemporal information and property/kind information was considered.

To this purpose, the animal model used was the chick of the domestic fowl (*Gallus gallus*) which belonging to a precocious species made it possible to investigate spontaneous innate abilities and to control for experiential variables due to the possibility to manipulate rearing conditions. I took advantage of the process of filial imprinting in all the experiments, exposing chicks for the first days of life to artificial objects which permitted to manipulate variables such as colour, size and shape. Then, chicks' spontaneous tendency to approach the larger group of imprinting and food stimuli was exploited as a behavioural tool (Rugani et al., 2009; Regolin et al., 2010) as well as the chicks' behaviour of rejoining imprinting objects once hidden by walking toward the position where the objects were seen disappear (e.g. Regolin et al., 2005; Vallortigara et al., 1998).

The experimental paradigm used was designed from Xu and Carey (1996) procedure and adapted to the species investigated. The chick underwent testing in a circular arena with two identical screens placed in two different spatial locations. Groups of events comprising different numerosity of stimuli were shown to the chicks (i.e. each group of event consisted either in one or more presentations of one stimulus or group of stimuli). At the end of stimuli presentation, the chick was set free in the arena and could approach one or the other screen. The assumption is that because chicks approach the larger quantity of imprinting objects they would choose the screen that hid the larger number of stimuli, irrespective of the number of presentations.

A first series of experiments (Exp.1 – Exp. 6) investigated chicks' ability to use property and spatiotemporal information. In Experiment 1 chicks' capability to use property information provided by colour, size or shape was assessed using a Sequential presentation test in which birds faced a comparison between two different objects presented once each *Vs.* one same object presented twice. In this way, an equal number of events was shown to the chick comprising a different number of objects. Chicks approached the screen that concealed two objects (differing for Colour, Size or Shape) as compared to the screen whereby a same object had been presented twice (such screen could therefore either hid one object or two identical ones). This result, though, can be explained in terms of use of property information for object individuation. An alternative explanation would be to account for chicks' behaviour in terms of a preference for the more varied group of stimuli. This hypothesis was ruled out in Experiment 2, in which a new group of chicks did not prefer two different to two identical stimuli when these were simultaneously presented.

Chicks used spatiotemporal information for object individuation, correctly individuated up to three objects simultaneously presented (Exp. 3).

Experiment 4 aimed to assess whether increasing the number of events would affect the use of property information: a 3 *Vs*. 2 events comparison was tested. If chicks would compute three presentations of the same object as more than two presentations each involving a different object, they would have approached the screen hiding only one object. On the other hand, if they individuated the actual number of objects using property information they would have approached the screen that hid two different objects. Results revealed that chicks did not show a preference for either group of objects. This lack of choice could be due to some limits in the process of objects individuation or to chicks' exposure during imprinting to objects identical in groups of three, hence becoming familiar to the fact that three identical objects can be encountered. Furthermore, both sets might be considered as correct because chicks had been exposed during imprinting familiarization to similar sets of objects (i.e. three yellow and three green objects). It seems that chicks compensate between the tendency to approach the larger number of presentations (i.e. one single object presented three times) and the smaller number of presentations but comprising two different objects. Differences in colour make more plausible that two different individuals are involved (i.e. two different objects presented once each).

Experiment 5 chicks were designed to shed light on this issue. Chicks were exposed to imprinting stimuli all differing from one another in their individual features, making it unlikely that subjects computed a repeated presentation of the same individual objects as many identical distinct individual objects. When birds were tested in a 3 *Vs.* 2 events comparisons (i.e. one individually distinct stimulus presented three times *Vs.* two different individually distinct stimuli presented once each), chicks chose the screen hiding the two distinct objects. Chicks therefore showed able to use property information provided by a peculiar feature for individuating the larger number of objects irrespective (and against) the number of events witnessed.

In all the previous experiments chicks were proved to be able to use property as well as spatiotemporal information to individuate objects to which they were exposed for the first two days of life. Towards the process of filial imprinting chicks have developed social attachment to those objects learning their features and forming a recognition memory. Thus, they demonstrated to possess the ability to spontaneously use property information at three days of life after familiarization following exposure to the objects. But since newborn chicks are able to move and feed independently, it is plausible they would soon, in the natural environment, face objects of which they have never had experience. Therefore, the aim of Experiment 6 was to determine whether previous, even though controlled, experience is needed for individuating objects. The purpose of this experiment was to investigate whether chicks make spontaneously use of property information also when provided by novel colours as compared to those used during familiarization. Chicks tested in a 2 *Vs.* 2 events comparison (i.e., one object presented twice *Vs*. two different objects each presented once), appeared to use property information provided by novel colour (which they had never seen before) for object individuation, choosing the screen hiding two different objects.

The second series of experiments (Exp. 7 - Exp. 10) was designed in order to deal with the issue regarding the phylogenetic development of the ability to use property/kind information for object individuation. This research represents the first attempt of studying such topic in a bird species.

Because chicks are precocious birds, able to follow the mother hen and the siblings and to feed independently soon after birth, I assumed as plausible that they may precociously exhibit some competences concerning the categories of objects most crucial to their survival. Feeding and social behaviours certainly constitute the two main domains in the chick's early life. Thus, food and social stimuli were used. Both categories are crucial, though they imply different behavioural responses, and plausibly, different levels of motivation are associated with either domain. For this reason, Experiment 7 and Experiment 8 investigated under which conditions chicks would be equally motivated to respond to food or to social stimuli. Chicks were presented with a 2 *Vs.* 2 stimuli comparison (two social stimuli *Vs.* two food stimuli, both elements in a set being simultaneously presented). If chicks would choose any of the two groups of stimuli, their behavior would likely indicate a preference for that category of objects. No preference would be displayed under conditions in which no bias is present towards one category of object.

Lack of interest for the two attractors could also explain lack of preferential choice. This possibility was ruled out beforehand: only chicks that promptly responded to both stimuli categories, which was established through an Imprinting Motivational test and a Food Motivational test, underwent the actual test.

Chicks exposed to yellow balls (which would constitute the imprinting stimuli) and fed four mealworms overall during the two days of rearing, in a simultaneous presentation of two food stimuli *Vs.* two imprinting stimuli, preferred the screen hiding food stimuli (Exp. 7). Manipulating several variables, such as increasing the attractiveness of the imprinting stimuli (i.e. the balls were red in colour), decreasing the number of mealworms supplied (i.e. two) and performing test on the second day of life (which are the crucial hours for triggering social attachment towards the process of filial imprinting and they still have nourishment for the residues of yolk sack), chicks did not preferentially choose between a group of two social stimuli and a group of two food stimuli, each set being simultaneously presented, even though they had proved to be motivated to respond to both type of stimuli beforehand (Exp. 8).

Lack of choice was considered to indicate equal motivation towards food and social attractors and therefore the key experiment for investigating property/kind information provided by those two categories of objects was designed employing same rearing conditions (Exp. 9). Chicks were tested with an identical number of events (i.e. two events) in a comparison between one stimulus presented twice *Vs.* two different stimuli each presented once (though in one case only one object was involved, whereas in the other case two objects were involved).

The assumption was that if chicks used property/kind information they should approach the screen hiding two different objects, because the difference in property/kind would suggest it more likely the presence of two distinct objects (i.e. of a larger group). Chicks individuated objects using property/kind information provided by imprinting and food items approaching the screen hiding two different objects. The alternative explanation that chicks may have chosen the group comprising an imprinting and a food object because they simply preferred a more varied group of stimuli has been ruled out in Experiment 10 through a simultaneous presentation of two different objects *Vs.* two identical objects. Under such conditions, chicks did not chose above chance either group of stimuli.

This research demonstrated that property, spatiotemporal as well as property/kind information is precociously available and spontaneously used by a bird species.

Employing an event-mapping task, infants at around ten months of age succeeded in the is-it-one-or-two task only when they were presented with one humanlike object (i.e. a doll's head) *Vs.* one inanimate (e.g., a motorcar) or one animate but non-humanlike (i.e. dog-like) object (Bonatti et al., 2001). This evidence suggested the existence in our species of a system for recognizing (at least) humans, animals and objects/inanimate things earlier available than a system for recognizing property-differences within the same category (e.g. a duck-toy *Vs.* a car-toy as in Xu & Carey, 1996). In my research the procedure for investigating object individuation employed mostly social stimuli such as imprinting objects that are very likely treated as animate objects by young chicks (see Mascalzoni et al., 2010). Moreover, the second series of experiments demonstrated that a similar mechanism can be present for individuating stimuli in other, beyond social, domains, such as food.

Differently from human and non-human primates, previous experience with the physical world seems not as crucial for chicks' spontaneous use of property information.

Monkeys use shape differences only after familiarization with shape-different objects (Mendes et al., 2011), and, for infants, the age in which colour and pattern are used for object individuation decrease if they were given previously specific experience with the objects: the colour or the pattern needed to be experienced as predictive of objects' function (Wilcox & Chapa, 2004). On the contrary, three-day-old chicks demonstrated to individuate objects using a novel property information (i.e. the novel colour of the stimuli), which they had never experienced before. Because chicks belong to a precocious species, it is likely that they develop species-specific abilities crucial for surviving in the natural environment soon after hatching, and in this sense, they are probably endowed with a mechanism that allows to successfully face unknown elements present in the environment. In contrast, mammalian species because of parental care requirements could develop this ability later combining maturation of the nervous system with experience.

Chicks were also able to use property/kind information provided by social and food stimuli. It seems that chicks possess rudimental representations of object concepts of different categories, at least of social and food and using them in object individuation. Those are probably the most crucial categories of objects to their survival. Nonetheless, the objection regarding the possibility that chicks used property information instead of kind differences for individuating objects could be put forward. Imprinting and food stimuli differed also for their physical-features and it may be that chicks rely on this information for succeeding in the task and not necessarily on kind information. However, social and food attractors imply very different behavioural responses which make it unlikely that chicks encode and use perceptual differences independently from the conceptual ones.

These results contribute to our understanding of object representation with evidence coming from a class of animals never tested before and, for the first time, from very young individuals. Domestic chicks, which belong to a precocial species, seem to be able to use spatiotemporal as well as property and property/kind information for object individuation at two or three days of life. Moreover, these data extend evidence obtained with pre-verbal infants, non-human primates and dogs, and permit to conclude that this ability can be independent of language and, apparently, of specific experience. They also support the hypothesis that those abilities evolved early in phylogenesis, as birds and mammals separated about 300 million years ago from the ancestral stem reptiles (Roger & Andrew, 2002) or, alternatively, that they evolved independently in mammals and birds as a result of homoplasy. In this sense, data obtained from the newly hatched chicks, or from other non-human mammalian species, would contribute to the issue of core knowledge systems providing evidence that object individuation ability, as part of the core system for representing object, is shared with non-human animals and it is present, at least in chicks, at few days of life, having deep roots in phylogeny.

The present comparative research contributes also to the validation of animal models for future neurobiological investigations thanks to the very well known neuroanatomy and neurophysiology of the species *Gallus gallus*.

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APPENDIX

The mean response times (seconds) for Imprinting Motivational Test and Food Motivational Test for each experiment (and then for each condition when present) are reported in the table.

MOTIVATIONAL TESTS	Imprinting Motivational test	Food Motivational test
Experiment 7	Mean= 36.549 sec sem= 8.597	Mean= 88.000 sec sem= 13.134
Experiment 8	Mean= 15.610 sec sem= 5.450	Mean= 81.916 sec sem= 16.032
Experiment 9	Mean= 14.000 sec sem= 2.293	Mean= 57.680 sec sem= 9.434
‘social x 2 *Vs*. social - food’ condition	Mean= 10.620 sec sem= 2.605	Mean= 41.666 sec sem= 5.682
‘food x 2 *Vs*. food - social’ condition	Mean = 17.115 sec sem= 3.578	Mean= 72.461 sec sem= 16.646
Experiment 10	Mean= 31.092 sec sem= 2.701	Mean= 91.428 sec sem= 8.528
‘social+social *Vs*. social+food’ condition	Mean= 31.314 sec sem= 4.276	Mean= 80.000 sec sem= 10.691
‘food+food *Vs*. food+social’ condition	Mean= 30.871 sec sem= 3.467	Mean= 102.857 sec sem= 12.950