Behavioral and Neuronal Category Representations in the Carrion Crow

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Abbreviations

DVR	dorsal ventricular ridge
IPS	intraparietal sulcus
MVL	*Mesopallium ventrolaterale*
NC	*Nidopallium caudale*
NCL	*Nidopallium caudolaterale*
NFL	*Nidopallium frontolaterale*
NIL	*Nidopallium intermedium laterale*
VIP	ventral intraparietal area
OPT	principle optic nucleus of the thalamus
PFC	prefrontal cortex
TPO	area temporo-parieto-occipitalis

Zusammenfassung

Ein Tier kann schnell und angemessen auch auf neuartige Reize reagieren, wenn es in der Lage ist, verschiedene Umweltreize sinnvoll zu kategorisieren. Verhaltensstudien der letzten Jahrzehnte haben gezeigt, dass Vögel zu komplexen Kategorisierungsleistungen fähig sind. Die zugrunde liegenden neuronalen Mechanismen wurden allerdings erst rudimentär untersucht. Die vorliegende Doktorarbeit beinhaltet eine Reihe von Verhaltens- und neurophysiologischen Untersuchungen an Rabenkrähen (*Corvus corone corone*), die grundlegende Erkenntnisse über die Physiologie von Kategorisierungsleistungen liefern.

In Bezug auf auditorische Kategorisierungsleistungen habe ich Rabenkrähen darauf trainiert, Schallsignale anhand der Richtung ihrer Frequenzänderung zu kategorisieren. Sie speicherten diese Informationen als offene Kategorien im Arbeitsgedächtnis und konnten so neuartige Stimuli sofort richtig zuordnen. In weiteren Studien konnten Krähen visuelle Informationen in einem aktiven Arbeitsgedächtnis gegen Störreize schützen und Gesichter von Krähen und Menschen unterscheiden, jedoch ohne Gesichter als spezifische Kategorie wahrzunehmen.

Zur Aufklärung der neuronalen Grundlagen habe ich Einzelzellableitungen im *Nidopallium caudolaterale* (NCL) von Rabenkrähen während kontrollierter komplexer Kategorisierungsaufgaben durchgeführt. Neuronen im NCL kodierten sowohl spontane als auch erlernte komplexe Kategorien. Sie reagierten ohne vorheriges Zahlentraining der Krähen auf diskrete Anzahlen, was auf ein angeborenes Zahlenverständnis hindeutet. Darüber hinaus kodierten die Zellen erlernte abstrakte Längenkategorien und passten sich flexibel an geänderte Kategoriezugehörigkeiten an. Schließlich habe ich Krähen darauf trainiert, ihre subjektive Entscheidung über das Vorhandensein oder Fehlen eines visuellen Reizes anzuzeigen. Die beiden Wahrnehmungsmöglichkeiten wurden von separaten Populationen von NCL Neuronen kodiert, die somit das neuronale Korrelat für das Wahrnehmungsbewusstsein der Krähen bildeten.

Zusammenfassend haben die in meiner Doktorarbeit enthaltenen Studien grundlegende Erkenntnisse über die Kategorisierungsleistung von Rabenkrähen geliefert und deutliche Gemeinsamkeiten der neuronalen Verarbeitung von Kategorien im NCL von Krähen und im Präfrontalkortex von Primaten gezeigt.

Abstract

Categorization is the key to simplification of the numerous stimuli which an animal encounters in a complex environment. It enables an animal to react fast and appropriately to all sorts of stimuli, even to novel ones. Behavioral studies conducted during the last decades show that birds master even most complex levels of categorizations. However, knowledge about the neuronal mechanisms that underlie this ability are scarce. My thesis includes a series of behavioral and neurophysiological experiments on carrion crows (*Corvus corone corone*) aimed at elucidating categorization capabilities in this bird species.

Addressing auditory categorization abilities, I trained carrion crows to categorize auditory stimuli based on the direction of frequency modulation. The results show that crows possess flexible categorical working memory to maintain high-level auditory category information and that they formed open-ended auditory categories which allowed them to immediately group novel stimuli correctly. Furthermore, we showed that crows use active working memory to protect visual information against interference and that they can discriminate images of crow and human faces but do not seem to represent faces as special categories.

To explore how neurons in the nidopallium caudolaterale (NCL) represent category-related information, I conducted single unit recordings while crows performed controlled behavioral protocols. NCL neurons represented spontaneously present as well as learned complex magnitude categories. We found that neurons in the NCL of numerically naïve crows responded to discrete numerosities even though they were not relevant to the task, suggesting that crows possess an innate 'sense of number'. Furthermore, NCL neurons encoded learned arbitrary categories of continuous spatial quantity (i.e. line length) and adapted flexibly to changed behavioral demands. Lastly, I trained carrion crows to report their subjective percept about presence or absence of a visual stimulus and showed that discrete populations of NCL neurons actively encoded the two perceptual states, thereby constituting the neural correlate of conscious subjective perceptions.

Taken together, the results of the included studies add further insights on the categorization abilities of crows and show striking similarities of category processing between NCL and the primate prefrontal cortex.

I Synopsis

1. Introduction

1.1. Categories and concepts

Living in a complex environment requires an efficient strategy to simplify the huge variety of surrounding stimuli. Categorization is the key to reduce the endless number of stimuli in an animal's environment to a smaller number of representations which share the same meaning. Grouping stimuli that require the same behavioral response provides a major advantage: it endows animals to react faster and to instantly adapt even when encountering a novel situation. Since the ability to categorize is a beneficial strategy in various situations, it is found in many species across animal kingdom.

Some categorical responses are innate, i.e. do not require previous learning. For example, crickets (*Teleogryllus oceanicus*) respond categorically to sounds according to their frequencies (promising either a conspecific or a predator) with a sharp behavioral change between moving towards or away from the source (Wyttenbach et al., 1996). Similarly, female lactating house mice (*Mus musculus*) respond categorically to ultrasonic sounds (typically produced by their pubs) showing searching behavior (Ehret and Haack, 1981). However, most categorization abilities are achieved through learning. For example, the calls of various bird species (Potvin et al., 2018). The alarm calls of black-capped chickadees (*Poecile atricapilla*) and Sibirian jays (*Perisoreus infaustus*) convey information about the size and threat of a predator (Templeton et al., 2005; Griesser, 2009). The structure of the chick-a-dee call of Carolina chickadees (*Poecile carolinensis*) conveys diverse information about the emitter and its environment (Freeberg, 2008), and American goldfinch (*Spinus tristis*) females recognize individual males based on their flight calls and respond specifically to the calls of their respective mates but not to other males (Mundinger, 1970). Likewise, mammals learn to interpret predator alarm calls. Suricates (*Suricata suricatta*) produce distinct alarm calls that convey information about predator category and risk (Manser, 2001) to which conspecifics respond accordingly (Manser et al., 2001). Similarly, vervet monkeys learn to respond appropriately to both, their conspecifics' and another species' alarm calls (Seyfarth and Cheney, 1990).

1.1.1. Levels of categories and concepts

The term category is used for the group of items which belong together, whereas a concept is the underlying knowledge or mental representation that enables categorization behavior (Zentall et al., 2002; Lazareva and Wasserman, 2017). The first definition of conceptual behavior suitable for comparative animal experimentation was proposed by Keller and Schoenfeld in 1950 (Wasserman, 2016; Lazareva and Wasserman, 2017). They proposed that an animal behaves conceptually if it responds similarly to members of one class and differently to members of other classes. However, this does still not exclude simple rote learning. Therefore, this definition was extended by Wasserman et al. (1988). These authors added that an animal must also transfer the same behavior immediately to novel (discriminably different) instances. An animal that fulfills these criteria demonstrates that it can form open-ended categories. This competence is very beneficial in everyday life because it gives sensory information a behavioral meaning (Freedman et al., 2003) and allows an animal to react fast and appropriately to a novel stimulus when it classified it correctly into a before learned category. Moreover, concepts can be distinguished based on their complexity into similarity-based perceptual concepts, nonsimilarity-based concepts and abstract relations, a distinction outlined in the next paragraph (Herrnstein, 1990; Zentall et al., 2002; Lazareva and Wasserman, 2017).

Similarity-based and nonsimilarity-based concepts

Items of a class that share a perceptual similarity, such as a physical feature, are regarded as similarity-based perceptual concepts (Zentall et al., 2002; Lazareva and Wasserman, 2017). This could be simple features like color (wavelength) or shape, or in the case of auditory stimuli the frequency of a sound. At this basic level of categorization, members of a category have a high perceptual similarity, whereas there is low perceptual similarity among members of different categories (Rosch and Mervis, 1975; Lazareva and Wasserman, 2017).

In contrast, in nonsimilarity-based concepts items are grouped by their meaning and do not necessarily need to share perceptual similarities (Herrnstein, 1990; Lazareva and Wasserman, 2017; Pusch et al., 2022). For instance, an apple and a banana, although not sharing similar physical appearance, can be both grouped together as fruits and therefore form a class of edible objects on a superordinate level. Besides this grouping into linguistic categories, items can

be grouped into learned functional equivalence classes according to a shared behavior or outcome; for example, all items which require the same response or are associated with the same amount of reward (Astley et al., 2001; Lazareva and Wasserman, 2017).

The seminal study by Herrnstein and Loveland (1964) in which pigeons discriminated pictures containing human beings paved the way for an intense investigation of categorization behavior in birds and other animals. They demonstrated that pigeons are able to discriminate pictures which contained humans from pictures which did not, and that they are able to generalize this behavior to novel images without prior learning. Later, it was shown that pigeons can learn various other categories, e.g. naturalistic categories like trees vs. no-trees (Herrnstein et al., 1976; Vaughan, 1988), water vs. no-water (Herrnstein et al., 1976), birds vs. mammals (Kendrick et al., 1990) as well as artificial stimuli like shoes or planes (Wasserman et al., 2015). In addition, pigeons demonstrated that they can learn up to sixteen different categories simultaneously (Bhatt et al., 1988; Wasserman et al., 2015) and can switch flexibly between categorizing a stimulus either according to its basic (car, chair, flower, person) or superordinate level (natural, artificial) (Lazareva et al., 2004). Further, pigeons can be trained to group stimuli into functional equivalence classes based on a common trained response (Wasserman et al., 1992), an associated amount or probability of reinforcement, or different delays until receiving a reinforcement (Astley et al., 2001; Astley and Wasserman, 1999).

The first corvids that have been examined for categorical skills were blue jays (Cyanocitta cristata). They were successfully trained to respond to photographs which contained a certain moth (i.e. prey) and showed generalization to novel images (Pietrewicz and Kamil, 1977). In a follow-up study these jays demonstrated again successful generalization abilities (Real et al., 1984). They were trained on two types of leaf silhouettes produced by different caterpillar species due to their feeding. After training with only one exemplar of each type, the jays correctly classified novel images of damaged leaves into the two classes.

Abstract relations

The most complex type of concepts are abstract relations, which are based on the relation between stimuli or even between concepts, such as same-versus-different and cardinal numbers (Herrnstein, 1990; Lazareva and Wasserman,

2017; Pusch et al., 2022). Although pigeons can be trained on basic relations, they show limitations in understanding abstract relations. Pigeons can perform same-versus-different tasks, but the training procedure determines whether they are able to transfer the learned rule also to novel stimuli (Edwards et al., 1983; Wright et al., 1988; Katz and Wright, 2006). In a simultaneous same-different discrimination task, pigeons were successfully trained to report whether the sixteen items in a display were all the same or all different. However, they did not transfer the previously learned rule to the situation when the stimulus display showing different items was changed so that only one of the sixteen items was different (Gibson et al., 2006). In another study, they were required to report whether a dot was inside or outside a curve, but they needed extensive training to perform the task (Herrnstein et al., 1989). Thus, pigeons can learn some abstract rules, but only if the training procedures are adequate (Herrnstein, 1990; Pusch et al., 2022).

Among birds, corvids (crows, ravens, magpies and jays) are thought to be cognitively superior to other birds and demonstrate complex cognitive abilities which match those of nonhuman primates (Emery and Clayton, 2004). Corvids use and manufacture tools (Hunt, 1996; Weir et al., 2002; Bird and Emery, 2009; Rutz et al., 2016; Kabadayi and Osvath, 2017), plan for the future when caching food for later consumption (Balda and Kamil, 1992) and take the social context during caching into account (Emery and Clayton, 2001; Bugnyar and Heinrich, 2005; Dally et al., 2006; Bugnyar et al., 2016). Scrub jays (Aphelocoma californica) even remember not only where they have hidden food but also distinguish between 'what' and 'when' suggesting an episodic-like memory (Clayton and Dickinson, 1998; Clayton et al., 2001). The cognitive similarities of corvids and primates make it worthwhile to further investigate these birds in order to gain comparative insights on the principles of higher cognitive abilities (Clayton and Emery, 2015). In the past, carrion crows (*Corvus corone corone*) demonstrated their ability to learn tasks of high complexity. They successfully performed a variety of tasks requiring abstract categorization abilities, such as rule-based same-different tasks (Veit and Nieder, 2013; Veit et al., 2014), unimodal and cross-modal associations (Veit et al., 2015; Moll and Nieder, 2015) and numerosity matching (Ditz and Nieder, 2015; 2016b). The current thesis exploits the crows' intelligence to address the physiology of categorization capabilities in these birds.

1.2. Topics included in this thesis

My thesis presents a range of studies which provide insights into the categorization ability of carrion crows. These categorization capabilities comprise categories that are useful for individual recognition, discrete and continuous magnitudes and categorical subjective percepts. As a significant extension of previous studies, my thesis specifically elucidates the neuronal mechanisms underlying explicit abstract categorization in crows.

1.2.1. Categorization abilities for individual recognition

Recognition of individual conspecifics can be beneficial for species living in social groups (Mateo, 2004; Tibbetts and Dale, 2007). It requires associating various features to an individual. These convey social information, such as gender and hierarchy rank within a social group, and allow recognition of mate and kin (Dale et al., 2001). Recognition of individuals has been reported from a wide range of animals, including mammals, birds, reptiles, fish and invertebrates (Dale et al., 2001; Leopold and Rhodes, 2010). Similar to other birds, corvids differentiate between specific individuals and adapt their behavior depending on the identity of the conspecific (Dally et al., 2006; Bird and Emery, 2008; Bugnyar, 2011; Massen et al., 2015). Moreover, corvids (and mockingbirds) demonstrate heterospecific recognition when responding differently to humans that they associate with a former negative experience (Levey et al., 2009; Marzluff et al., 2010). In birds, the capacity to recognize others relies mainly on auditory and visual features (Thorpe, 1968; Falls and Brooks, 1975; Weary and Krebs, 1992; Ryan and Lea, 1994; D'eath and Stone, 1999; Brecht and Nieder, 2020). Particularly songbirds rely on auditory information and recognize individuals based on auditory features (Thorpe, 1968; Brecht and Nieder, 2020). For instance, ravens (*Corvus corax*) differentiate between familiar and unfamiliar individuals based on their vocalizations alone and remember their relationship to familiar individuals even three years later (Boeckle and Bugnyar, 2012). Similarly, carrion crows (*Corvus corone*) can discriminate conspecifics based on vocalizations (Wascher et al., 2015), and jungle crows (*Corvus macrorhynchos*) can be trained to discriminate the contact calls of different individuals in a Go/No-Go task (Kondo et al., 2010). This ability requires assigning auditory stimuli a categorical meaning. Whether crows can categorize controlled acoustic stimuli into auditory categories and maintain such auditory categories active in working memory is explored in Wagener and Nieder (2020).

Apart from auditory features, birds were shown to distinguish others based on visual characteristics like plumage (Whitfield, 1986; Brown and Dooling, 1992; Dale et al., 2001), but also facial cues (Nakamura et al., 2003; 2006; Marzluff et al., 2010). Faces offer many relevant social information about others especially for mammals and humans in particular with their elaborated facial musculature (Todorov et al., 2008; Leopold and Rhodes, 2010). The ability to recognize faces is thought to rely on a specialized configural (holistic) processing, which is why face recognition is affected when the face is presented upside down (Collishaw and Hole, 2000; Maurer et al., 2002). Exploring whether crows can discriminate faces and whether faces are perceived as a special category was object of one study included in my thesis (Brecht et al., 2017).

1.2.2. Discrete and continuous magnitudes

The concept of number is a particularly abstract categorization (Nieder et al., 2002). This is because sets showing the same number of items are grouped together. The only aspect that matters is the stimulus entity itself; all sensory features (e.g. size, location, shape, etc.) of the individual items are completely irrelevant. The ability to judge quantity is reported from various species in the animal kingdom, e.g. insects (Dacke and Srinivasan, 2008), fish (Agrillo et al., 2011; Bisazza et al., 2014), amphibians (Uller et al., 2003; Stancher et al., 2015), birds (Pepperberg, 1994; Emmerton and Renner, 2006; Roberts, 2010; Scarf et al., 2011; Ditz and Nieder, 2015; 2016a) and mammals (Meck and Church, 1983; Brannon and Terrace, 1998; Nieder et al., 2002; Beran, 2007; Vonk and Beran, 2012). The finding that animals can discriminate quantitative information without explicit training led to the idea of a 'sense of number' which describes an intuitive ability to perceive the number of items in a set (i.e. its 'numerosity'). Based on this 'number sense' animals can understand numerical quantity without relying on language (Dehaene, 2001; Viswanathan and Nieder, 2013; Nieder, 2016). One of the studies included in my thesis provides neurobiological evidence for an innate 'number sense' in crows (Wagener et al., 2018).

In order to assess numerosity nonverbally, two representational systems are discussed, the 'object file system' and the 'approximate number system'. In the object file system, it is thought that mental 'markers' are assigned to the individual items in a set. This mechanism can only represent a limited number of up to four items due to the restricted number of 'markers' (Nieder, 2005;

Feigenson et al., 2004). This set-size limit is considered to be a signature of the object file system and has been reported for rhesus monkeys (Hauser et al., 2000) and human infants (Feigenson et al., 2002).

In contrast, the approximate number system has no upper boundary. It allows animals to estimate numerical quantity by representing it akin to a continuous magnitude (Meck and Church, 1983; Carey, 2001). The magnitude which is assigned to each number is proportional to the number of items in the set (Carey, 2001). The approximate number system follows the Weber's law. In order to perceive a difference between two stimuli, the amount of the just noticeable difference (ΔI) relative to a reference stimulus intensity (I) has to be proportional to this intensity I. In other words, the Weber-fraction ($\Delta I/I$) is a constant (Weber, 1850; Nieder and Miller, 2003). The minimal amount by which two stimulus intensities need to differ so that they are perceived as being different is called the 'just noticeable difference' (Nieder and Miller, 2003). Fechner extended Weber's law. He proposed that the relationship between the subjective perception (S) and the physical stimulus intensity (I) is proportional to the logarithm of the intensity (with k being a constant) which leads to Fechner's law (S = k*log(I)) (Fechner, 1860). Because both laws share fundamental commonalities, they are sometimes combined as the 'Weber-Fechner law'.

Weber's law predicts a numerical distance effect and a numerical size effect for quantity discrimination. The distance effect implies that two magnitudes are easier to discriminate the greater the difference is between them. For instance, a numerosity 2 is easier to discriminate from a numerosity 8 than from numerosity 3. The size effect on the other hand, indicates that it is equally difficult to discriminate magnitudes with the same ratio (e.g. 2 from 4 and 4 from 8). Put differently, the discrimination of two magnitudes of the same distance is more difficult for larger magnitudes (2 from 4 vs. 12 from 14). The numerical distance effect and the numerical size effect are clear signatures of the approximate number system. This system has been reported for different bird species, such as chicks (Rugani et al., 2013), pigeons (Emmerton and Renner, 2006; Roberts, 2010; Scarf et al., 2011) and crows (Ditz and Nieder, 2016a), as well as for teleost fishes (Agrillo et al., 2014; DeLong et al., 2017), salamanders (Krusche et al., 2010), nonhuman primates (Brannon and Terrace, 1998; Nieder and Miller, 2003; Beran, 2007), human infants (Feigenson et al., 2004) and adult humans (Merten and Nieder, 2009).

The first suggestive evidence for a neuronal correlate of number was demonstrated in anesthetized cats by a small proportion of neurons in the posterior association cortex which responded to specific numbers of presented auditory and visual stimuli independent of modality (Thompson et al., 1970; Nieder, 2021a). Later, high proportions of single neurons that encode discrete numerosities have been found in the prefrontal and posterior parietal cortex of behaving rhesus monkeys (Nieder et al., 2002; Nieder and Miller, 2004), the medial temporal lobe of humans (Kutter et al., 2018) and the nidopallium of carrion crows (Ditz and Nieder, 2015; 2016b). The neuronal mechanisms underlying numerical competence show strong correspondence in the very distantly related birds and primates (Nieder, 2020). Numerosity is encoded by neurons which respond to individual (preferred) numerosities which is characteristic for a labeled-line code (Nieder and Merten, 2007; Ditz and Nieder, 2015). Interestingly, the tuning functions are best described on a logarithmic number line as predicted by the Weber-Fechner law (Piazza et al., 2004; Ditz and Nieder, 2015; Kutter et al., 2018; Nieder et al., 2002; Nieder and Miller, 2003). Although both birds and mammals demonstrate comparable numerical abilities, it seems likely that their numerical competences emerged independently through convergent evolution, since their respective brain regions containing numerosity-encoding neurons originate from different parts of the pallium (ventral in birds and dorsal in mammals), and reptiles, being close relatives of birds, rather rely on continuous quantity instead of discriminating discrete numerosities (Nieder, 2021a). In contrast to discrete numerosities, non-numerical magnitudes vary along a continuous scale. To complement our knowledge on discrete quantity representations, one study of the current thesis therefore explores how conventional categories of continuous magnitudes, such as line length, are represented by neurons in the crow brain (Wagener and Nieder, 2023).

1.2.3. Categorical subjective percepts

Sensory (primary) consciousness is the awareness of subjective experiences that can also be reported (Edelman, 2001; Nieder, 2021b). Exploring whether sensory consciousness is present in animals is difficult because even complex behaviors can be produced without conscious awareness (Nieder, 2022). However, using ambiguous stimuli subjective percepts can be explored. For example, reversible images like the rabbit-duck illusion which elicits either the perception of a rabbit or a duck although the image stays constant, or binocular rivalry occurring

when the eyes see dissimilar images at the same time can be used (Nieder, 2021b).

Sensory stimuli presented with varying intensity near perceptual threshold (e.g. the frequency of a vibration or the intensity of a visual stimulus) evoke bistable perceptions as well. In order to report the conscious experience about a sensory stimulus, the continuous feature needs to be translated into a binary categorical percept (e.g. 'present' or 'absent'). Forced-choice tasks can be used to measure the perception threshold of a sensory stimulus by requiring a certain behavioral response according to whether a stimulus had been perceived or not. While this is easy for very low and very high stimulus intensities, it becomes difficult for intermediate (near-threshold) intensities; here, the two percepts alternate from trial to trial. The probability of perceiving an intermediate stimulus as present or absent is described by signal detection theory and depends on the threshold level (Green and Swets, 1966). Responses can be classified as 'hit' (correct 'yes' response to a present stimulus), 'miss' (erroneous 'no' response to a present stimulus), 'correct rejection' (correct 'no' response to stimulus absence) and 'false alarm' (erroneous 'yes' response to stimulus absence).

Ambiguous sensory information are also suitable to explore the neural correlate of consciousness (NCC). Neurons which encode the subjective percept (e.g. 'present' versus 'absent') in response to identical stimuli reflect the sensory consciousness (Nieder, 2021b). So far, neurophysiological studies have been conducted only in human and nonhuman primates with the assumption that the NCC relies on a layered cerebral cortex (Myerson et al., 1981; Leopold and Logothetis, 1996; de Lafuente and Romo, 2005; Gelbard-Sagiv et al., 2018). However, assuming the cerebral cortex to be the prerequisite of conscious perception would implicate that birds are not endowed with the ability of subjective experiences because they lack a layered neocortex. This seems highly unlikely considering the cognitive abilities of birds and particularly corvids (Emery and Clayton, 2004; Güntürkün et al., 2017). Therefore, we designed an experiment including visual stimuli of various intensities between zero (not visible) and clearly detectable in order to explore the neuronal correlate of subjective percepts in the avian brain (Nieder et al., 2020). The neuronal response to physically identical near-threshold stimuli which elicit opposite internal percepts (and thus reflecting sensory consciousness) has been found in the prefrontal and premotor cortex of macaque monkeys (de Lafuente and

Romo, 2005; Merten and Nieder, 2012). In one study of my thesis, we show evidence for the neuronal correlate of categorical subjective percepts in the NCL of crows (Nieder et al., 2020).

Previous studies mainly suggest that the NCC relies on neurons which actively encode the presence of a stimulus and otherwise stay silent (de Lafuente and Romo, 2005; Quiroga et al., 2008; Van Vugt et al., 2018). Studies reporting an active encoding of stimulus absence are rare. Neurons which increased their firing rate when a stimulus was not perceived have been found in macaque monkeys (Merten and Nieder, 2012) and humans (Pereira et al., 2021). In one of the studies included in my thesis, we investigated whether the perception of stimulus absence is represented by a distinct neuronal population also in birds (Wagener and Nieder, 2024).

1.3. The avian brain

The neurophysiological studies which are part of my thesis confirm the similar processing mechanisms in birds and the very distantly related mammals. Birds and mammals share a common ancestor, the stem amniote. However, their lineages diverged already around 320 million years ago (Dos Reis et al., 2015). The classical view on the avian brain was based on Ludwig Edinger's view that evolution is a linear progress of increasing complexity with new brain areas being added with each new vertebrate group until the reaching the apex represented by humans (Emery and Clayton, 2005; Jarvis et al., 2005; Güntürkün, 2012). According to Edinger, the avian telencephalon (which consists of pallium and subpallium) was mistakenly thought to be mainly derived from the subpallium and therefore homolog to the basal ganglia of mammals, and only a small part from the pallium (Reiner et al., 2004; Emery and Clayton, 2005; Shimizu, 2009; Olkowicz et al., 2016). This was further supported by the fact that the avian telencephalon appears anatomically similar to the mammalian basal ganglia as a nuclear mass organized into clusters instead of layers (Reiner et al., 2004; Shimizu, 2009). Because of this misinterpretation, birds were for a long time thought to produce only reflexive and instinctive behavior (Reiner et al., 2004; Emery and Clayton, 2005; Shimizu, 2009; Olkowicz et al., 2016). First doubts about this came up in the 1960s (Karten, 1969). Only the subpallium turned out to be homologous in birds and mammals and it was estimated that it constitutes against previous assumptions only a quarter of the avian

telencephalon (Jarvis et al., 2005). Recent findings confirmed that in songbirds, the subpallium constitutes only 10-22% of the telencephalon volume whereas the rest corresponds to the pallium (Olkowicz et al., 2016). In 2002, most structures of the avian brain were renamed by the Avian Brain Nomenclature Consortium to now reflect their pallial origin (Reiner et al., 2004; Jarvis et al., 2005).

Although the rest of the telencephalon of both birds and mammals shares a pallial origin as suggested by molecular similarities (Puelles et al., 2000), the long time of independent evolution resulted in substantially differently organized brains which is especially pronounced in the telencephalon. The most striking difference between the avian and mammalian pallium is that in birds it is not organized in a laminated fashion (Shimizu, 2009). In mammals, the largest part derived from the pallium is the six-layered neocortex (besides also non-laminated structures) (Puelles et al., 2000; Shimizu, 2009). The information processing among the cortical layers was thought to underlie complex behavior in mammals (Calabrese and Woolley, 2015). Therefore, a layered cortex was for a long time seen as the critical basis of complex cognitive abilities (Reiner et al., 2004; Jarvis et al., 2005). In birds, however, the largest part of the telencephalon consists of the DVR (dorsal ventricular ridge) which forms areas that are segregated into nuclei (i.e. entopallium, mesopallium, nidopallium and arcopallium) and the laminated Wulst (hyperpallium) (Shimizu, 2009; Briscoe and Ragsdale, 2018). Similar processing circuits as in the mammalian layered cortex have been found in birds which suggests that these circuits not necessarily have to be organized into layers to guide complex behavior and that canonical microcircuits were already present in a common ancestor (Wang et al., 2010; Shanahan et al., 2013; Calabrese and Woolley, 2015). Very recently, analyzing the fiber architecture of the avian pallium, a cortex-like circuitry was detected in the sensory DVR and Wulst, however not in the associative (NC) and motor areas (Stacho et al., 2020).

1.3.1. Emergence of category representations in the visual processing hierarchy

A hypothesis of how category representations in the avian brain emerge in the course of hierarchically organized brain areas was recently proposed (Güntürkün et al., 2021; Pusch et al., 2022). Birds possess two distinct visual pathways which are homologous but functionally different to those of mammals

(Karten et al., 1973; Koenen et al., 2016). The thalamofugal pathway is in birds rather subordinate, although it corresponds to the in mammals dominant geniculostriate pathway (Shimizu, 1993). It proceeds via the principle optic nucleus of the thalamus (OPT) to the visual Wulst which is suggested to be the avian equivalent of the primary visual cortex (Medina and Reiner, 2000). On the other hand, the tectofugal pathway which is homologous to the mammalian extrageniculocortical pathway is considered to be the dominant visual pathway in birds (Pusch et al., 2022). It conveys visual information from the retina through the optic tectum and the nucleus rotundus to the entopallium (Karten and Shimizu, 1989). The entopallium is involved in shape and motion perception which is processed in distinct subdivisions (Nguyen et al., 2004; Stacho et al., 2016). Complex category information is not yet present at this stage (Azizi et al., 2019), however, neurons in the entopallium of pigeons reflect basic stimulus-reward associations (Anderson et al., 2020).

The entopallium is further reciprocally connected to surrounding associative areas, like NFL (nidopallium frontolaterale), NIL (nidopallium intermediale pars lateralis), MVL (mesopallium ventrolaterale) and TPO (area temporo-parieto-occipitalis) (Krützfeldt and Wild, 2005). In these areas, in contrast to mammalian visual associative areas, the previous functional separation of shape and motion processing is no longer present (Stacho et al., 2016). In the past years, these areas have been investigated electrophysiologically in order to find category-specific responses. Spontaneous basic category representations without prior category training have been found in NFL differentiating between pictorial and grating stimuli (Koenen et al., 2016) and in MVL distinguishing between ‘animate’ and ‘inanimate’ real-world objects (Azizi et al., 2019). The categorization success of a linear classifier which was used to categorize the population activity of MVL neurons increased with the number of included neurons (Azizi et al., 2019; Güntürkün et al., 2021; Pusch et al., 2022). Further, MVL neurons, similar to entopallium, distinguished pictures based on their associated reward (Anderson et al., 2020). Another study revealed a preference for intact over scrambled objects of the neuronal population in the NIL of pigeons (Clark et al., 2022). Thus, although some category-related responses could be found in these associative areas, more complex category representations seem to be absent at this stage.

1.3.2. The NCL as the center of categorization abilities in birds

The hierarchically highest processing area in the avian brain is the NCL (nidopallium caudolaterale). It is the central basis for the generation of executive functions which allow flexible behavior and functionally resembles the primate prefrontal cortex (PFC) (Güntürkün, 2005). It was first described by Mogensen and Divac (1982) which showed that lesions in this area produce the same behavioral deficits as lesions in the PFC. This was confirmed by further behavioral studies using lesioning (Hartmann and Güntürkün, 1998; Diekamp et al., 2002) and blocking of NCL (Kalenscher et al., 2003). The equivalence of NCL and PFC was further verified by neurochemical studies focusing on the dopaminergic innervation (Divac and Mogensen, 1985; Wynne and Güntürkün, 1995). Both share a similar organization of dopaminergic innervation (Güntürkün, 2005) receiving comparable input from the ventral tegmental area and the substantia nigra (Gaspar et al., 1992; Waldmann and Güntürkün, 1993; von Eugen et al., 2020). Anatomically, the NCL can be distinguished from neighboring structures by its distribution of several receptor types (Herold et al., 2011).

In order to generate flexible behavior, both NCL and PFC serve as multimodal integration stages between sensory and dopaminergic input and motor output (Güntürkün, 2005). Both receive highly processed multimodal sensory input from secondary and tertiary areas and project onto premotor areas (Kröner and Güntürkün, 1999; Güntürkün, 2005; Nieder, 2017a). Functionally the NCL resembles PFC in mediating higher cognitive abilities (Güntürkün, 2021). For example, among those category-related functions are encoding of stimulus-reward relations (Kirsch et al., 2009; Anderson et al., 2020), abstract rules (Veit and Nieder, 2013; Veit et al., 2014), uni- and multimodal associations (Veit et al., 2015; 2017; Moll and Nieder, 2015; 2017) and numerical categories (Ditz and Nieder, 2015; 2016b; Kirschhock et al., 2021). These anatomical and functional analogies to the primate PFC argue for the NCL being the center of complex categorical representations in birds.

2. Results

2.1. Behavioral performance of crows in categorization tasks

2.1.1. Auditory categorization

Assigning auditory stimuli a categorical meaning is a useful ability when differentiating other individuals based on their vocalizations, particularly for songbirds. In contrast to visual working memory, less is known about categorical working memory in the auditory domain. Therefore, in this study, we explored the ability of crows to classify auditory stimuli and memorize sound categories (Wagener and Nieder, 2020). Two crows performed a delayed match-to-category task with auditory stimuli. We used controlled pure tones which differed in their direction of frequency modulation (upward vs. downward sweeps). In a first step, the crows were trained on a set of three upward and three downward modulated sample stimuli. They learned to match these stimuli to the corresponding test stimulus with the same direction of frequency modulation. Once they reached stable performance, we started with the generalization test to investigate whether the crows were able to assign novel stimuli to the learned categories. Generalization is the next level after learning categories by rote. Responding in the same way to all members of a category, and therefore also to those never encountered before, is the key feature of open-ended categorization. In the generalization test, while the crows continued to perform the task with the training set, we presented novel sounds randomly interleaved within the training set. We used four classes of novel probe stimuli: three types of pure-tone FM sweeps (linear, logarithmic and quadratic modulation) and frequency-modulated segments of bird vocalizations. To exclude effects of learning, we only analyzed responses to the first presentation of each probe stimulus.
Both crows mastered the generalization test with the three classes of pure-tone FM sweeps. One crow additionally reached significant categorization performance with the bird vocalizations. These results reveal that crows can form open-ended auditory categories and memorize auditory category information to bridge a delay period between sample presentation and required response.

2.1.2. Impairment of working memory by visual distractors

The ability to group stimuli into meaningful classes and maintain such representations active over brief time intervals relies on categorical working memory (Miller et al., 2018). In this study, we aimed to figure out whether carrion crows

use active working memory as opposed to simple short-term memory in order to maintain relevant information which is needed to solve a visual delayed match-to-sample task (Wagener et al., 2023). To that aim, we modified a classical delayed match-to-sample task by introducing interfering stimuli in the middle of the delay period between sample presentation and the test phase. To solve the task, the crows needed to maintain relevant information about the sample stimulus and protect it against distracting information throughout the entire delay period. We hypothesized that sample representations would be strongly impaired if crows maintain them in passive short-term memory. However, if crows possess active working memory, they could filter out distracting information and thus protect relevant information from being overwritten. We used three different visual interfering stimuli: a neutral image (gray circle) as reference condition, the initially shown sample stimulus repeated, or a distractor image that was subsequently presented as nonmatch stimulus in the following test phase.
Both crows performed the task proficiently with all three types of interfering stimuli. Relative to the neutral interfering stimulus, the repeated presentation of the sample stimulus within the delay improved performance (both in terms of accuracy and reaction time). In contrast, the presentation of the nonmatch distractor caused a mild performance decay. The results indicate that the crows maintained the relevant sample information in active working memory which enables them to protect it against distraction.

2.1.3. Face recognition

Recognition of conspecifics relies on the discrimination of their individual features; however, it is unknown whether birds use visual cues for conspecific recognition (Brecht and Nieder, 2020). Demonstrating that faces as a visual category are processed differently compared to other visual stimuli would suggest that faces play an important role for birds. An indicator for that can be the so called face inversion effect which in humans is described as an impairment of face recognition when the faces are presented upside down (Farah et al., 1995; Collishaw and Hole, 2000; Maurer et al., 2002). We aimed to explore such a potential face inversion effect suggesting a specialized processing of faces in carrion crows (Brecht et al., 2017). For that, we trained two crows to discriminate profiles of crow faces as well as human faces and corresponding control images (fish and house interior), either presented upright or inverted, in a match-to-sample task.

The crows performed above chance level (50%) for each stimulus category indicating that they are able to discriminate the individual stimuli within a category. However, both crows neither showed a face inversion effect for crow faces nor for human faces. Irrespective of the absence of a face inversion effect, we found that crows had a better performance discriminating crow faces than human faces. As a control of the experimental procedures, we tested twenty human participants in the same task with slightly adapted timing (shorter sample stimulus presentation and longer delay) to prevent performance ceiling effects. In contrast to crows, humans had a face inversion effect for human faces thus verifying that the used methods were suitable to detect an effect.

2.2. Neuronal representations of categories in the NCL of crows

2.2.1. Neural correlate of magnitudes

Spontaneous encoding of numerosity in the crow NCL

From previous studies we know that crows can be successfully trained to perform a delayed match-to-numerosity task (Ditz and Nieder, 2015; 2016b). Their behavioral performance as well as the neuronal correlates of numerosities in the NCL resemble those of monkeys (Nieder et al., 2002; Nieder, 2018). However, following the idea of a 'number sense' (Dehaene, 2001), crows might have an intuitive understanding of the number of items in a set and thus a neural representation of numerosities even without numerosity training as it was described for monkeys (Viswanathan and Nieder, 2013). In this study, two numerically naïve crows performed a delayed match-to-sample task which simply required them to match the color of the dots in numerosity stimuli (Wagener et al., 2018). The stimuli provided behaviorally irrelevant numerosity information unnecessary to solve the task. In a generalization test, we tested whether the crows indeed did not rely on numerosity information. To that aim, we inserted trials in which the dot color of all stimuli was black (pure numerosity stimuli). In these trials, the crows performed at chance level, showing that they did not use the numerosity information to solve the task.

Although the crows did not actively perform a numerosity task and were never trained on numerosity stimuli before, we found neurons in the NCL which encoded the number of dots in the presented stimuli. The neurons responded to the stimuli in the same way as it has been shown for numerically trained crows in the previous studies: neurons showed a peak in firing rate to

the preferred numerosity and a decay of activity to neighboring numerosities (Ditz and Nieder, 2015; 2016b). This indicates that numerosity-selective neurons already exist in the crow brain without prior training and confirms the hypothesis that crows possess an innate concept of numbers.

Encoding of continuous magnitude in the crow NCL

In this study, we investigated the response of NCL neurons to learned magnitude categories (Wagener and Nieder, 2023). We used line length as an example of an abstract spatial magnitude. In contrast to numerosities, line length is continuous and has no discrete entities. We trained two crows on a match-to-category task which required them to group horizontal lines according to their length. A sample stimulus of a certain line length was presented and after a delay the crows had to respond to the test stimulus which showed a line length of the same category. The category boundary was arbitrary and divided the six different stimuli into the two categories 'short' (the three shortest line lengths) and 'long' (the three longest line lengths). The behavioral performance displayed the characteristic shape of similarly high accuracy for all stimuli of the same category and a sharp change across the category boundary.

We recorded the activity of NCL neurons and found neurons which responded selectively to the individual categories already during the presentation of the sample stimulus, but even more so during the delay. The activity of these neurons showed the same characteristics evident in behavioral performance. The discharge rates were similar to all line lengths within a category and changed sharply across the category boundary.
Several decoding analyses confirmed categorical representations at the level of the neuron population. Transforming the activity of the category-selective neurons into state space resulted in trajectories that displayed neuronal population activity to the six different line lengths in the course of a trial. The trajectories representing within-category neuronal activity were closer in state space, indicating similar representations. However cross-category activity was significantly more distant in state space, suggesting distinct representations. Applying a k-means clustering algorithm revealed that during the delay, the optimal number of clusters into which the individual trials can be divided, reflected the two learned categories 'short' and 'long'.
In a next step, we focused only on the delay activity and explored the category coding capability of the entire population of recorded neurons (not preselected

for category-selectivity). In order to compare the neuronal discharge rates to the different line lengths, we created a correlation matrix displaying the similarity of the responses to each pair of line lengths. This confirmed that even without any preselection for category-selectivity, the population responded similarly to members of the same category and differently across categories. Cross-category activity differences vanished in trials in which the crows responded incorrectly, indicating that the neuronal representations are behaviorally relevant for the crows to group the line lengths into the correct categories.
Another population analysis confirmed that all members of a category are treated similarly and at the same time differently to members of the other category. We tested the classification performance of a support vector machine (SVM) classifier which was trained with only one line length of either category. Irrespective of which stimuli were used for training, the classifier correctly grouped the discharge rates of the remaining stimuli into the correct categories, indicating that the neuronal response to each line length, no matter whether it was far or close from the category boundary, was predictive of the other category members.

In order to explore the effect of category learning on the behavior and the neuronal responses, we retrained one crow to categorize the same line lengths based on new arbitrary boundaries. Now the crow grouped the six stimuli into three different categories ('short', 'medium' and 'long'). The behavioral performance was similarly high as in the previous two-category task and again displayed the characteristic similar performance to the line length within a category and a sharp change across categories.
We applied the same population decoding analyses as for the two-category task. The k-means clustering algorithm now revealed that the activity of the category-selective neurons during the delay can be optimally divided into the newly three trained categories. Also, the correlation matrix and a SVM classifier confirmed that, after retraining, the entire population of recorded neurons encoded the three new categories by displaying the characteristic category-specific response pattern, i.e. responding similarly to members of the same category and differently to members of other categories. Comparing the neuronal activity in the two tasks shows that NCL neurons encode learned continuous magnitude categories in a behaviorally relevant way and flexibly adapt to the required task conditions.

2.2.2. Neural correlate of categorical subjective percepts

In this study, we aimed to identify the neural correlate of sensory consciousness – brain activity in the NCL which reflects the subjective report about whether or not the crows had perceived the presented stimulus (Nieder et al., 2020). Two crows were trained to perform a stimulus detection task which required them to report their binary categorical decision about presence ('yes') or absence ('no') of the visual stimulus. The stimulus was a gray square presented at different intensities ranging from zero (stimulus absent) to an intensity which was clearly detectable. In contrast to assigning certain behaviors for the two different response options, we used rule cues which indicated whether the birds were required to make a Go-response or withhold from responding in order to report their decision on a trial-by-trial basis, thus preventing them from preparing confounding motor actions.

The behavioral performance plotted against stimulus intensity formed a typical psychometric function, showing that zero and highest intensities had been reliably perceived as absent or present, respectively, whereas intermediate intensities around perceptual threshold had been detected as being present in approximately half of the trials. We classified the intensities into 'no stimulus' (stimulus absent), 'near-threshold' (stimulus present but faint) and 'supra-threshold' (stimulus present and salient). Near-threshold stimuli eliciting alternating percepts despite containing identical physical information enable the investigation of subjective experience. The behavioral responses of the crows were grouped according to signal detection theory into 'hit' (correct 'yes' response to a present stimulus), 'miss' (erroneous 'no' response to a present stimulus), 'correct rejection' (correct 'no' response to stimulus absence) and 'false alarm' (erroneous 'yes' response to stimulus absence) (Green and Swets, 1966).

We found a proportion of single NCL neurons which encoded the subjective experience of the crows. The activity of these neurons reflected categorically the subjective percept (stimulus present or absent), especially shortly before making the required response, irrespective of the true presence or absence of the stimulus.

To investigate the binary categorical responses at the population level, we used a SVM classifier to discriminate 'yes' and 'no' responses based on the neuronal activity. We trained the classifier on firing rates in near-threshold trials in which the crows responded 'yes' and 'no' equally often to the identical near-threshold stimulus intensity. Thereafter, the classifier was able to assign the crow's report also to firing rates from no-stimulus trials and supra-threshold trials, indicating that the neuronal activity to near-threshold stimuli conveyed sufficient information about the subjective report. The same was true for firing rates in near-threshold trials, when the classifier was trained on 'yes'-responses in supra-threshold and 'no'-responses in no stimulus trials. Overall, these data show that neuronal activity in the NCL corresponds with the crows' subjective percepts.

In a follow-up study, we investigated whether both perceptions about stimulus presence but also absence are encoded actively (Wagener and Nieder, 2024). Indeed, two different neuronal populations reflected the crows' subjective percept by an increased activity to 'yes' responses ('yes'-neurons) and 'no' responses ('no'-neurons), respectively. Particularly during the delay period between stimulus presentation and response phase, we found similar neuron numbers and encoding properties for both classes suggesting equal representation of both subjective states.

3. Discussion

In the past, several experiments have shown that carrion crows can perform complex behavioral tasks, such as rule-based delayed match-to-sample (Veit and Nieder, 2013; Veit et al., 2014), visual and multimodal association (Veit et al., 2015; 2017; Moll and Nieder, 2015; 2017), and match-to numerosity tasks (Ditz and Nieder, 2015; 2016b; Kirschhock et al., 2021). The key to solve these tasks is the ability to form concepts about abstract rules. We exploited this ability of crows to conduct experiments targeting the categorization behavior and gain further insight into its underlying neuronal mechanisms in birds. We showed evidence for a categorical auditory working memory (Wagener and Nieder, 2020) and demonstrated that crows use active working memory to protect visual information against interference (Wagener et al., 2023). When testing facial categories, crows discriminated images of crow and human faces but did not seem to process faces as special categories (Brecht et al., 2017). On the neuronal level, we found category-selective neurons existing in the NCL already without prior category training and that crows possess an innate 'sense of number' (Wagener et al., 2018). Further, NCL neurons encoded learned arbitrary categories of continuous spatial magnitudes and can be trained to flexibly encode changing category demands (Wagener and Nieder, 2023). Finally, neurons in the crow NCL categorically reflected the subjective experience about presence or absence of a visual stimulus, constituting the neural correlate of consciousness (Nieder et al., 2020; Wagener and Nieder, 2024).

3.1. Categorization behavior of crows

3.1.1. Auditory categorization

Auditory features play a major role in the discrimination of individual conspecifics based on their vocalizations. Songbirds and also pigeons have been shown to be able to discriminate and classify various auditory stimuli (songbirds: Dooling et al., 1992; 1995; Burgering et al., 2019, pigeons: Murphy and Cook, 2008; Cook and Brooks, 2009; Brooks and Cook, 2010; Cook et al., 2016; Cook, 2017). And a few studies in European starlings showed auditory working memory in these songbirds (Zokoll et al., 2007; 2008; Comins and Gentner, 2010). However, these previous studies typically used a Go/NoGo procedure or forced choice task without a delay period requiring a temporal storage between stimulus presentation and response.

The delayed match-to-category task that we used satisfied two requirements. First, it required maintaining categorical information about an auditory stimulus in working memory to bridge the delay period (Wagener and Nieder, 2020). We showed that carrion crows possess flexible categorical auditory working memory by maintaining not only specific auditory stimuli but higher-level auditory category information. Second, by testing the crows with novel auditory stimuli, we demonstrated that they formed an open-ended concept of frequency modulation direction allowing them to immediately transfer the classification rules to novel sounds, irrespective of the frequency composition of the pure tones and partly even for complex sounds such as bird vocalizations. Together, this provides convincing evidence for true categorical auditory working memory in crows.

3.1.2. Lack of evidence for a special face category

Although corvids can recognize individual conspecifics (Dally et al., 2006; Bird and Emery, 2008; Bugnyar, 2011), it is not clear on which features they rely to do so. Some bird species were found to use facial cues for discrimination (budgerigars: Trillmich, 1976; Brown and Dooling, 1992, pigeons: Nakamura et al., 2003). Further, it was shown that crows indeed differentiate humans based on their faces. Jungle crows can be trained to categorize photographs of human faces according to their sex (Bogale et al., 2011) and wild American crows, after a negative experience (capture) with people wearing unique face masks, responded with stronger scolding behavior to the mask which was worn during the previous negative experience than to neutral masks, even after several years (Marzluff et al., 2010). Likewise, our results showed that crows can discriminate pictures of crow and human faces; however, we did not find that faces are treated in a specialized manner (Brecht et al., 2017). Similarly, pigeons as well seem to have no face inversion effect when discriminating human and monkey faces (Phelps and Roberts, 1994). This would suggest that birds in general do not perceive faces as a holistic special category. When recognizing others, birds might rather use features of the whole body. Pigeons were reported to categorize images of pigeon bodies (Nakamura et al., 2006). Thus, it would be interesting whether they and also crows display a body inversion effect, as it was shown for humans (Reed et al., 2003), however this was not yet tested.
The presence of a face inversion effect has been reported for some mammals (e.g. humans: Collishaw and Hole, 2000; Maurer et al., 2002; Brecht et al., 2017, monkeys: Wright and Roberts, 1996, sheep: Kendrick et al., 1996) and insects

(e.g. honeybees Dyer et al., 2005). A paper wasp species showed faster and more accurate learning for real conspecific faces than for manipulated faces and other images (Sheehan and Tibbetts, 2011). Thus, for some species at least, faces are special categories.

To support face processing, human and nonhuman primates have specialized 'face areas' in the temporal lobe (Perrett et al., 1982; Desimone et al., 1984; Kanwisher et al., 1997; Tsao et al., 2006). Single-neuron activity in these face patches of monkeys revealed that they contain almost exclusively face-selective neurons, suggesting specialized areas only for face recognition (Tsao et al., 2006). Activity in the individual face patches differs depending on the viewing conditions and neurons in one of the anterior face patches represent facial identity irrespective of the viewpoint (Freiwald and Tsao, 2010). Similarly, cells in the temporal cortex of sheep respond to sheep and human faces (Kendrick and Baldwin, 1987). In the medial temporal lobe of human patients, neurons were found that represented different categories of visual stimuli, such as faces, animals or specific objects (Kreiman et al., 2000). Some of these neurons termed 'concept neurons' respond selectively to different pictures of a certain individual person, their written name and in some cases also to the pictures of a closely related person (Quiroga et al., 2005). These neurons reflect abstract concepts via sparse coding, i.e. they show elevated activity to members of a concept and apart from that stay silent (Quiroga et al., 2008).
Some studies have attempted to find face-selective neurons in the visual system of birds, too. An imaging study revealed various brain areas in American crows, including nidopallium and mesopallium, being activated during perception of threatening and caring human faces, respectively (Marzluff et al., 2012). However, although exploring various brain areas in pigeons including the terminals from both visual pathways (Wulst and entopallium) and also higher order visual association areas (MVL, NFL, TPO), a convincing fraction of neurons being selective to pigeon faces in these regions was not found (Clark et al., 2019; 2022). Whether such specialized neurons exist in the NCL which receives projections from these association areas is still unknown. Images of bird faces have been used in a delayed match-to-sample task while recording from NCL neurons of crows, however a specialized neuronal population for faces was not reported (Veit et al., 2014). Thus, based on current evidence, special face processing modules seem to be absent in birds.

3.2. Neuronal category representations in the crow brain

3.2.1. Spontaneous category representations

Some categorical representations don't seem to require learning and experience. We found neurons in the crow NCL that encoded categorical numerosity information although the birds were never trained on numerosities before and the task did not require to use these (Wagener et al., 2018). Later, such numerosity-selective neurons have also been found in the NCL of untrained 10-days old domestic chicks (Kobylkov et al., 2022). These findings parallel results in monkeys in which numerosity-selective neurons were present in the PFC and VIP (ventral intraparietal area) of rhesus monkeys (Viswanathan and Nieder, 2013).
Numerosity training seems to enhance neuronal representations of number. The proportion of numerosity tuned neurons in naïve crows (12%) was smaller compared to the proportion in numerically trained crows (20%, Ditz and Nieder, 2015). Comparable amounts of numerosity-selective neurons have also been found in the VIP (13%) and PFC (14%) of numerically naïve rhesus monkeys (Viswanathan and Nieder, 2013). However, the quality of numerosity-selective responses, measured as the widths of the tuning curves, was similar for both naïve and trained crows, suggesting that numerosity training increases the proportion of selective neurons but does not change their coding properties. The numerosity-selective neurons in naïve as well as in trained crows were tuned to individual preferred numerosities (Wagener et al., 2018; Ditz and Nieder, 2015; 2016b), suggesting a labeled-line code for numerosity encoding. The same coding properties have been found in numerically naïve (Viswanathan and Nieder, 2013) and trained monkeys (Nieder et al., 2002; Sawamura et al., 2002; Nieder and Miller, 2004; Nieder, 2017b). The spontaneous emergence of numerosity-selective neurons is suggested to rely on inherent mechanisms of the visual system, since such units also emerged in a deep neural network model which was trained to classify natural images unrelated to numerosity (Nasr et al., 2019).

Other examples of spontaneous category representations, even though rather basic ones, have been reported from areas upstream to the NCL in birds. In the NFL of pigeons, a higher visual area receiving input from both, the tecto- and thalamofugal pathway, category-specific activity was found distinguishing between pictorial and grating stimuli (Koenen et al., 2016). Further, in MVL, the neuronal population distinguished between 'animate' and 'inanimate' real-

world objects (mainly driven by a distinction between 'human' and 'nonhuman' photographs within the 'animate' category) although it was not required by the task (Azizi et al., 2019). Thus, object-specific representations seem to gradually emerge along the visual pathways until full-blown categorical representations manifest in the NCL.

3.2.2. Representation of learned categories in the avian brain

New categorical behavior is mostly acquired through reinforcement learning. In mammals this is closely associated with the neuromodulator dopamine. Since NCL receives strong dopaminergic input, it is likely that category representation is shaped by dopamine signals (Güntürkün et al., 2018). Several studies provided evidence that dopamine signals in NCL affect reinforcement learning and working memory (Puig et al., 2014; Rose et al., 2009; Herold et al., 2008; 2012).
Two possible mechanisms could lead to the development of category-selective neurons. First, former nonselective neurons could develop category representations. Engel et al. (2015) proposed a cortical circuit model showing that category representations develop between sensory and decision layers by previously nonselective neurons becoming category-selective if fluctuations of their firing rates are correlated with behavioral outcome. Alternatively, already existing category-selective neurons could additionally encode novel categorizations. In an association experiment for which crows were trained to associate colorful images to certain pictorial stimuli, neurons in the NCL which encoded already learned familiar associations were found to represent also the associations of novel images after learning these by trial-and-error (Veit et al., 2015; 2017).

Rudimentary category representations of rather less complex categories seem to emerge first in the higher visual association areas NFL and MVL which receive input from the terminals of the two visual processing pathways (entopallium and Wulst) (Koenen et al., 2016; Atoji and Wild, 2012; Azizi et al., 2019). These findings suggest that higher visual areas pre-process visual information which is then projected onto NCL where highly complex representations of categories and concepts emerge (Güntürkün et al., 2018; Pusch et al., 2022).

Neurons in the NCL of pigeons were found to represent stimulus-reward relations by encoding visual stimuli based on their behavioral meaning in a

Go-/No-Go task (Kirsch et al., 2009) and based on their reward outcome in an S+/S- discrimination task (Anderson et al., 2020). In crows, specialized NCL neurons represent abstract rules in a rule-based delayed match-to-sample task (Veit and Nieder, 2013), encode visual stimuli categorically based on their respective associates (Veit et al., 2015; 2017) and encode discrete numerosities in match-to-numerosity tasks (Ditz and Nieder, 2015; 2016b; Kirschhock et al., 2021). Our results add to these the encoding of abstract continuous magnitude categories (Wagener and Nieder, 2023) and categorical subjective perceptions about presence or absence of a visual stimulus (Nieder et al., 2020), emphasizing once more the functional similarity between the avian NCL and the primate PFC (Güntürkün, 2005; Güntürkün et al., 2021).

Abstract and learned magnitude categories were encoded by NCL neurons displaying characteristic category-specific tuning functions (i.e. similar response to all members within a category and sharp difference in response to members of other categories), as well as by the entire population of recorded neurons in the NCL irrespective of any preselection for category-selectivity (Wagener and Nieder, 2023). This pattern of activation is reminiscent to findings in the primate PFC. In a seminal series of experiments, macaques were trained in a delayed match-to-category task to categorize morphed images according to the arbitrary categories 'cat' and 'dog', respectively (Freedman et al., 2001; 2002; 2003; Roy et al., 2010; 2014; Cromer et al., 2010; 2011). The neurons encoded the category information of the morphed stimuli by responding similarly to all stimuli within a category (e.g. 'dogs') and differently with a sharp change in activity to the stimuli of the contrary category (e.g. 'cats') (Freedman et al., 2001; 2002; Roy et al., 2010; Cromer et al., 2010).
Underscoring the plasticity of categorical representations in crows, we found that the activity in NCL was changed through learning. When one crow was retrained to categorize the same stimuli according to other category boundaries, the neuronal response in the NCL reflected clearly the newly learned categories (Wagener and Nieder, 2023). This indicates that NCL responses can be actively shaped based on task demands. Similar flexibility was reported for the PFC of monkeys (Freedman et al., 2001; 2002; Roy et al., 2010; Cromer et al., 2010), which emphasizes the functional similarity of the corvid NCL with the primate PFC.
It would be interesting to test whether discrete and continuous quantities are represented by the same or different neuronal populations in the NCL of birds

and whether both have the same encoding properties. To test, crows would need to be trained to perform a match-to-category task including both dot arrays and line lengths. This experiment was performed in macaque monkeys and revealed mainly distinct neuron populations that either encode numerical or continuous spatial quantity, but rarely both magnitudes in the intraparietal sulcus (IPS), a region which contains high proportions of numerosity-selective neurons (Nieder, 2005) and the PFC (Tudusciuc and Nieder, 2007; 2009). Similar to the encoding of numerosities, the neurons had the same tuning properties for the spatial quantities (line lengths), showing a peak in activity for the preferred line length and a progressive drop-off for more remote lengths. This is in line with a common processing mechanism proposed for numerical quantity and the continuous magnitudes space and time (Walsh, 2003).

The ability to consciously report the subjective experience about a sensory stimulus requires brain activity representing the abstract categorical perception about stimulus presence or absence. Using a stimulus detection task which required crows to report their categorical perception of a visual stimulus that varied along a continuous intensity scale, we showed the respective neuronal correlate of this percept reflected by neurons in the NCL (Nieder et al., 2020). These neurons responded similarly whenever the crow judged that it had seen (or not seen, respectively) the stimulus, irrespective of the presented intensity. Similarly responding neurons have been reported to exist in the PFC and other areas in the frontal cortex of monkeys (de Lafuente and Romo, 2005; Merten and Nieder, 2012; 2013). Our findings provide the first evidence for conscious perception in a nonmammalian species and suggest that a layered cortical architecture is not a prerequisite for sensory consciousness. Birds possess a cortex-like circuitry which exhibits similar processing dynamics to that of mammals and could therefore constitute the neural basis of sensory consciousness (Shanahan et al., 2013; Stacho et al., 2020; Güntürkün, 2021).
Further, and also consistent to the findings in the PFC of monkeys (Merten and Nieder, 2012), stimulus absence was encoded actively by neurons which increased their firing rate reflecting the ‘no’ response similarly to the neuronal population which encoded ‘yes’ responses about stimulus presence (Wagener and Nieder, 2024). Active representation of stimulus absence was recently described also in the context of numerosity. Neurons in the NCL of crows actively encoded empty set stimuli in the same manner as countable numerosities (Kirschhock et al., 2021).

Sensory neurons typically encode the presence of a stimulus by increasing their firing rate and otherwise remain at baseline activity (Hubel and Wiesel, 1959; 1962). Therefore, while neurons reflecting the subjective perception of stimulus presence can rely on activated sensory neurons, the representation of stimulus absence requires internal generation of categorical activity. The neuronal representation of stimulus absence as a distinct category might be an advantage for cognitively advanced animals constituting goal-directed behavior.

3.3. Conclusion

We showed that carrion crows form open-ended auditory categories and exhibit working memory for sound categories. They protected visual information in an active working memory against interference and can discriminate images of crow and human faces but do not seem to represent faces as special categories. Neurons in the NCL represented complex categories, whether spontaneously present or learned, in a behaviorally relevant way. Our findings suggest striking similarities of category processing between NCL and the PFC of primates. Overall, our results confirm and significantly extend previous assumptions on the substantial role of NCL mediating high-level cognitive functions.

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II Individual Studies

Statement of Contributions

My thesis comprises seven publications, which are summarized and discussed in the synopsis. The individual publications can be found on the following pages.

1. **Wagener, L.**, Nieder, A. (2020) Categorical auditory working memory in crows. *IScience*, 23(11), 101737.

   I designed the study together with A.N., performed the experiments and analyzed the data. Together with A.N., I interpreted the data and wrote the manuscript. A.N. supervised the study.

2. **Wagener, L.**, Rinnert, P., Veit, L., Nieder, A. (2023) Crows protect visual working memory against interference. *Journal of Experimental Biology*, jeb-245453.

   The study was designed by A.N. and L.V. Together with P.R. and L.V., I performed the experiments and analyzed the data. All authors interpreted the data. I wrote the manuscript together with A.N. A.N. supervised the study.

3. Brecht, K. F., **Wagener, L.**, Ostojić, L., Clayton, N., Nieder, A. (2017) Comparing the face inversion effect in crows and humans. *Journal of Comparative Physiology*, 203(12), 1017-1027.

   The study was designed by K.F.B., me, L.O. and A.N. I conducted the experiments together with K.F.B. I analyzed the data together with K.F.B. and A.N. All authors interpreted the data. K.F.B. and A.N. wrote the paper. A.N. supervised the study.

4. **Wagener, L.**, Loconsole, M., Ditz, H. M., Nieder, A. (2018) Neurons in the endbrain of numerically naive crows spontaneously encode visual numerosity. *Current Biology*, 28(7), 1090-1094.

   I designed the study together with H.M.D and A.N. I performed the experiments together with M.L. I analyzed the data and together with A.N., wrote the paper. A.N. supervised the study.

5. **Wagener, L.**, Nieder, A. (2023) Categorical representation of abstract spatial magnitudes in the executive telencephalon of crows. *Current Biology* 33(11), 2151-2162.

   I designed the study together with A.N. I performed the experiments and analyzed the data. Together with A.N., I interpreted the data and wrote the manuscript. A.N. supervised the study.

6. Nieder, A., **Wagener, L.**, Rinnert, P. (2020) A neural correlate of sensory consciousness in a corvid bird. *Science*, 369(6511), 1626-1629.

All authors designed the study. I and A.N. conducted the experiments. I analyzed the data together with P.R. and A.N. All authors wrote the paper. A.N. supervised the study.

7. **Wagener, L.**, Nieder, A. (2024) Conscious experience of stimulus presence and absence is actively encoded by neurons in the crow brain. *Journal of Cognitive Neuroscience* 36(3), 508-521.

   Together with A.N., I designed and conducted the experiment, analyzed the data and wrote the paper. A.N. supervised the study.

Study 1: Categorical auditory working memory in crows

Wagener, L., Nieder, A. (2020) Categorical auditory working memory in crows. *IScience*, 23(11), 101737.

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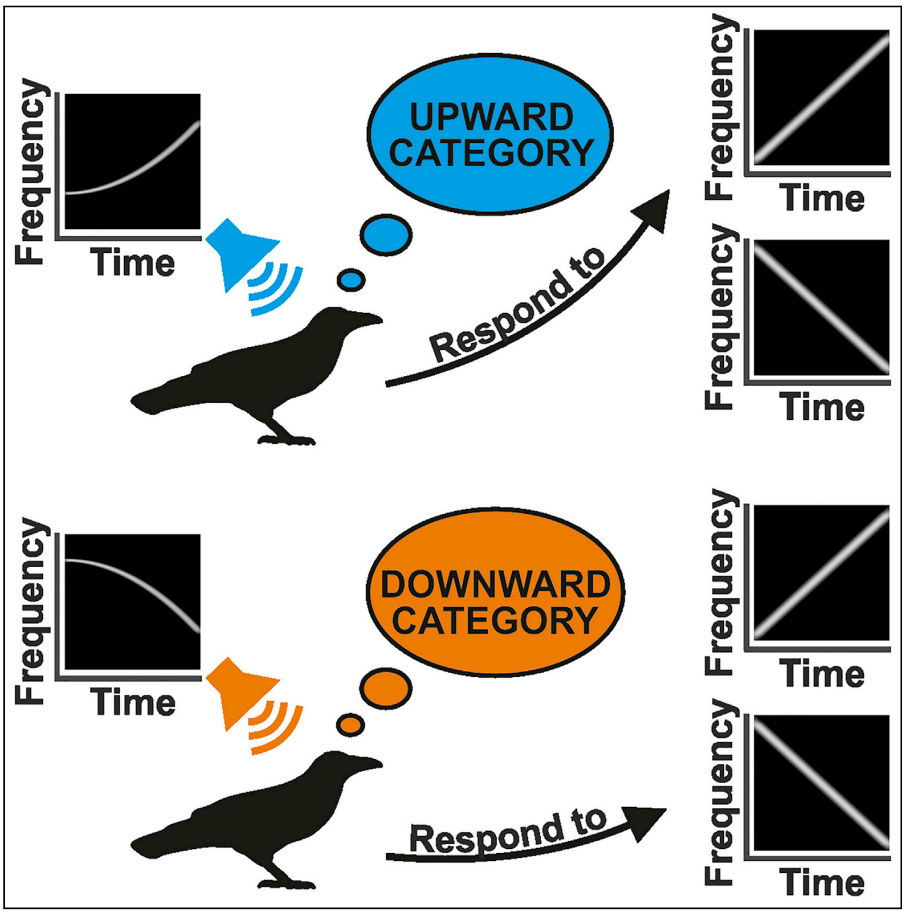
Categorical Auditory Working Memory in Crows



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HIGHLIGHTS

Crows performed a delayed match-to-category task with frequency modulated sounds

Crows classified novel sounds into upward or downward modulated sound categories

Crows showed sharp category boundaries and within-category generalization

Crows can actively memorize auditory perceptual categories for cognitive control

Wagener & Nieder, iScience *23*, 101737
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Categorical Auditory Working Memory in Crows

Lysann Wagener[1] and Andreas Nieder[1,2,*]

SUMMARY

The ability to group sensory data into behaviorally meaningful classes and to maintain these perceptual categories active in working memory is key to intelligent behavior. Here, we show that carrion crows, highly vocal and cognitively advanced corvid songbirds, possess categorical auditory working memory. The crows were trained in a delayed match-to-category task that required them to flexibly match remembered sounds based on the upward or downward shift of the sounds' frequency modulation. After training, the crows instantaneously classified novel sounds into the correct auditory categories. The crows showed sharp category boundaries as a function of the relative frequency interval of the modulation. In addition, the crows generalized frequency-modulated sounds within a category and correctly classified novel sounds kept in working memory irrespective of other acoustic features of the sound. This suggests that crows can form and actively memorize auditory perceptual categories in the service of cognitive control of their goal-directed behaviors.

INTRODUCTION

Categorical working memory, the ability to group sensory data into behaviorally meaningful classes and to maintain them active in working memory for a future goal, is key to intelligent behavior (Miller et al., 2018). It allows humans and animals to classify, memorize, and process sensory information efficiently. This enables humans and cognitively advanced animals to quickly adapt to new situations (Miller et al., 2003).

So far, categorical working memory in animals has primarily been demonstrated in the visual domain. In classical working memory tasks, monkeys and crows flexibly switch between remembered visual categories, such as "leftward versus rightward motion" (Zhou and Freedman, 2019), "cats versus dogs" (Freedman et al., 2001), or "same versus different" (Wallis et al., 2001; Veit and Nieder, 2013). However, whether categorical working memory is also found in the auditory domain is currently unknown.

This lack of knowledge about auditory categorical working memory is surprising because this cognitive capability is essential during goal-directed audio-vocal communication. In a telephone group call, for instance, we categorize speech signals as belonging to a specific individual and maintain this auditory category in working memory in order to match it to subsequent speech signals of the same speaker while following a conversation. Undoubtedly, also animals that rely on elaborate audio-vocal communication would benefit from this cognitive ability. Unfortunately, most animals are notoriously difficult to train on complex auditory tasks (Plakke and Romanski, 2016). Currently it is therefore rarely studied whether animals can actively maintain auditory categories in working memory (Tsunada et al., 2011).

As true vocal learners, songbirds face many challenges of acoustic communication with speaking humans (Mooney, 2009). To follow an audio-vocal communication, songbirds need to recognize communication partner's characteristics, such as sex, group membership, or identity (Wascher et al., 2015; Brecht and Nieder, 2020). In short, songbirds rely both on acute hearing and cognitive abilities to classify a multitude of raw acoustic stimuli and memorize this information across time (Nieder and Mooney, 2020). Indeed, songbirds are known to perceive sounds in a categorical way (Dooling et al., 1995; Burgering et al., 2019). In addition, they show working memory for auditory items comparable with humans (Zokoll et al., 2007; Comins and Gentner, 2010). However, whether birds can combine both capabilities to actively memorize auditory categories for future goal-directed behavior is unknown, and this capability is barely studied in animals in general. Here, we addressed this issue in carrion crows, a vocal corvid songbird

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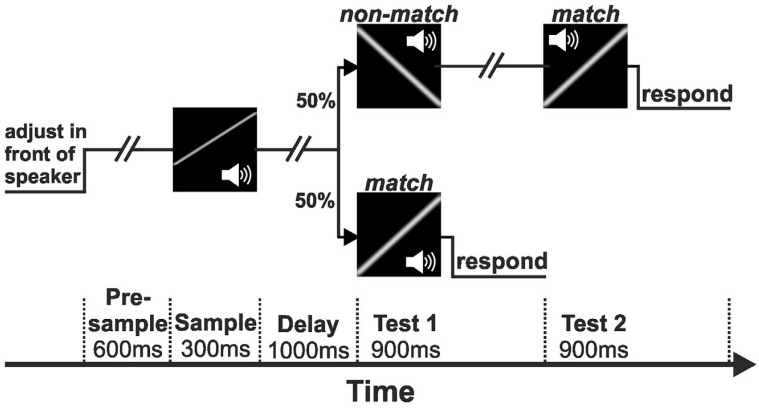

Figure 1. Task Design

The trial began when the crow adjusted its head in front of the speaker and screen (by entering an infra-red light barrier) in response to a central visual Go-cue displayed on the screen. After the crow had adjusted its head, the screen turned blank for the rest of the trial. A silent pre-sample period (600 ms) was followed by a frequency-modulated sample sound that was played for 300 ms. The sample was followed by a 1s silent delay and then by a choice (Test) sound (900 ms). *Lower trial end-sequence:* If the category (upward or downward FM) of Test1 matched that of the sample ("match" condition), the crow had to move its head and leave the infra-red light barrier to the Test1 sound within the 900 ms response time (shifted by 100 ms relative to Test-onset) to obtain a food reward. *Upper trial end-sequence:* If Test1 was a nonmatch ("non-match" condition), a match followed as Test2, which required a head movement for a reward. There were an equal number of match and nonmatch trials and they were randomly interleaved.

that can be trained on complex tasks (Nieder, 2017; Brecht et al., 2019; Nieder et al., 2020) requiring conceptual understanding and behavioral flexibility (Veit et al., 2015; Moll and Nieder, 2014; Smirnova et al., 2015; Ditz and Nieder, 2016a).

RESULTS

We trained crows on a delayed match-to-category task with sounds (Figure 1). In this task, the crows indicated whether a test sound was a categorical match to a previously presented and memorized sample sound. In each trial, the crows evaluated and maintained the direction of frequency modulation (FM) of the sample sounds in working memory to subsequently match them to the upward or downward modulated sound categories. Since individual trials presented varying sound combinations, the crows had to flexibly categorize what they heard on a trial-by-trial basis.

The crows were first trained to match six fixed FM sample stimuli ("training stimuli," three upward and three downward sweeps) to the upward or downward categories (Figures 2A and 2B). The frequency range of the upward and downward FM stimuli together covered the entire hearing range of crows (Jensen and Klokker, 2006). Once the crows reached reliable performance with these training sample stimuli, novel probe sample stimuli were occasionally inserted in the daily sessions (Figures 2C–2E), while the crows continued to discriminate the training stimuli as background task. Both crows performed 10 successive sessions with randomly interleaved training and probe stimuli.

For the training sample stimuli, crow O performed an average of 430 correct background trials per session (±52 STD, n = 10) and reached mean performance of 85.2% (±6.1% STD across sessions) (Figure 3). Crow G on average accomplished 426 correct background trials per session (±36 STD, n = 10), with a mean performance of 87.7% (±2.5% STD) (Figure 3). The average performance of both crows with the background stimuli in each daily session was significantly above the 50%-chance level (each binomial test, $p < 0.001$). Owing to the temporal succession of the matching test stimulus in the "match" versus "non-match conditions," both crows had a bias toward responding to test1, resulting in systematically higher performances during match trials (see separate data points for match and non-match performances in Figure 3). However, not only match but also all non-match performances separately were significantly above chance for both crows and all conditions (each binomial test, $p < 0.001$). The crows' mean performances for each of the six training sample stimuli was indifferent (each one-way ANOVA, $p > 0.05$).

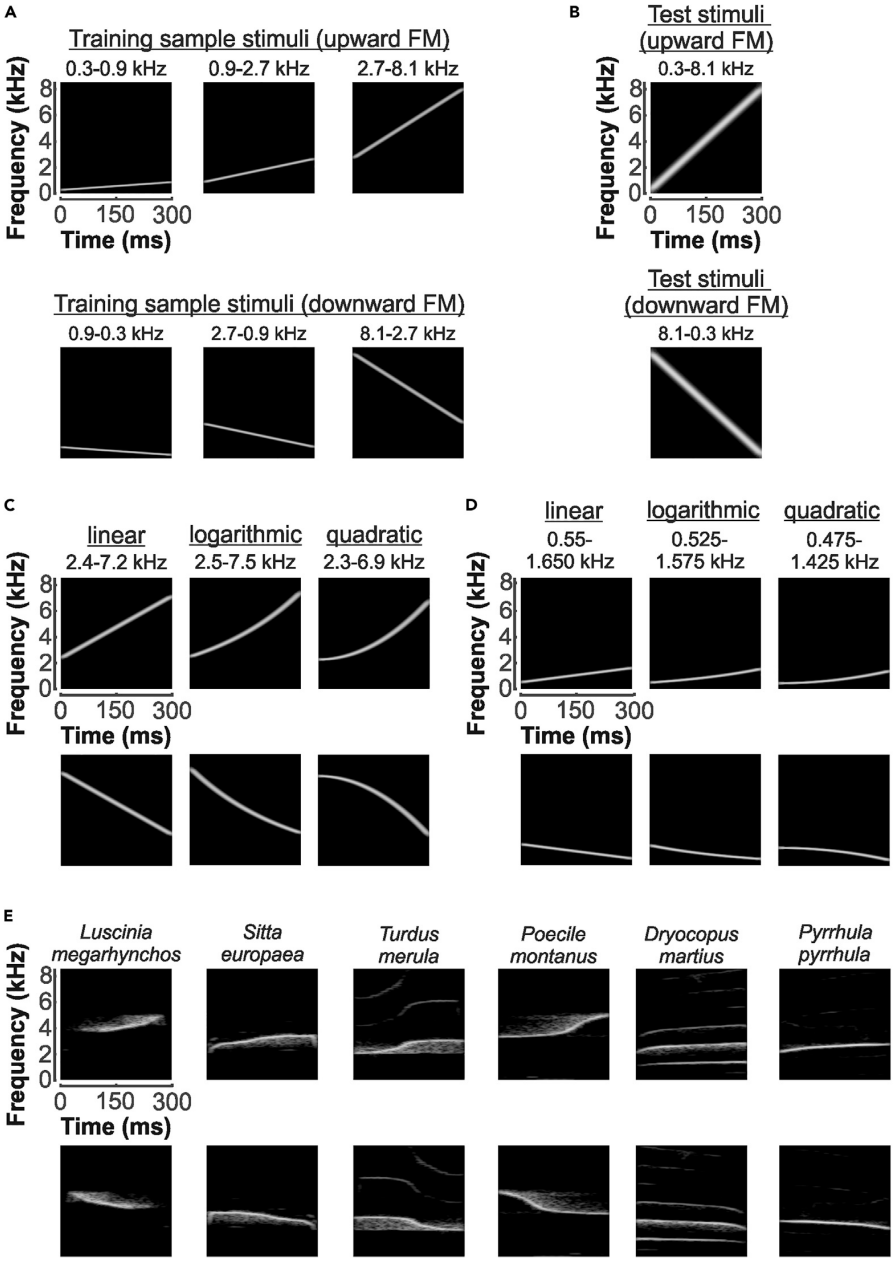

Figure 2. Auditory Stimuli Depicted as Sonagrams

(A) The familiar sample stimuli for training the crows were three upward and three downward linear FM sweeps.

(B) The same two upward and downward FM sweeps were used as test stimuli.

(C) Examples of new probe sample stimuli with frequency interval ratios of 3:1 (1.6 octaves). Linear, logarithmic, and quadratic sweeps in a high-frequency range are shown. Top row displays upward FM sweeps, bottom row shows the corresponding downward FM sweeps.

(D) Same layout as in (C), showing linear, logarithmic, and quadratic sample probe stimuli in a low-frequency range.

(E) Probe sample stimuli consisting of segments of bird vocalizations. Six representative examples of the 20 stimuli are depicted. Top row shows upward, bottom row the corresponding mirrored downward stimuli.

Next, we tested whether the crows could generalize novel FM sounds they had never heard before to the appropriate categories and thereby would demonstrate a conceptual grasp of sound categories. To that aim, we occasionally introduced novel probe sample sounds (12% of the trials) in the daily sessions with the training sounds (the remaining 88% of the trials). Four classes of novel probe sample sounds were presented: three classes of pure-tone FM sweeps with linear (where frequency changes linearly with time), logarithmic (where frequency changes logarithmically with time), and quadratic (where frequency changes quadratically with time) frequency trajectories, and frequency-modulated segments of bird vocalizations. The frequency interval ratios of the pure-tone probe sweeps were 2:1 (1 octave), 3:1 (1.6 octaves) (examples in Figures 2C and 2D), and 4:1 (2 octaves). The mean frequency interval ratio of probe bird vocalizations was 1.47:1 (around half an octave), on average (Figure 2E). The number of upward and downward-modulated probe stimuli was balanced. Because the goal was to test whether the crows could instantaneously transfer the FM categories without additional learning, we only analyzed responses to the first presentation of each unique probe stimulus.

Across all probe stimuli and classes, both crows showed a significant category transfer (each binomial test, $p < 0.001$, $n = 160$) (Figure 4). For all ten sessions together, crow O responded 80% (128/160 trials) and crow G responded 77% (123/160) correctly across all probe stimuli (which was comparable with the performance with training stimuli in crow O but significantly worse in crow G; binomial test, $p < 0.05$). To ensure that the transfer was made for each of the two categories, we analyzed the performance to upward and downward FM probes separately. Again, both crows performed well above chance level for both categories separately (each binomial test, $p < 0.01$, $n = 80$) (Figure 4). Crow O responded correctly in 81% and 79% of the trials presenting upward and downward FM probe stimuli, respectively. Crow G responded correctly in 68% and 86% of the trials presenting upward and downward FM probe stimuli, respectively. Again, not only match but also non-match performances separately were significantly above chance for both crows and all conditions (each binomial test, $p < 0.05$), except for one (downward for crow O, binomial test, $p = 0.059$).

Categorization is characterized by sharp category boundaries and within-category generalization. We first analyzed performance as a function of distance to the category boundary. The physical dimension for categorization of FM sounds into the perceptual "upward" and "downward" categories is the frequency interval ratio of the sounds. A frequency interval ratio of 1 (i.e., no change in frequency with time) demarcates the category boundary relative to which upward versus downward frequency-modulated sounds of increasing frequency interval ratio can be classified into the FM categories upward versus downward. Figure 5 depicts the crows' judgments of upward category as a function of the probes' frequency interval ratios. As expected for categorical behavior, the crows classified rising FM sounds into the upward category and falling FM sounds into the downward category, with an abrupt switch of performance at the category boundary. Performance for probe sweeps at high-frequency interval ratios (4:1, 3:1, and 2:1) (each binomial test, $p < 0.001$, $n = 30$ for ratios of 4:1 and 2:1, respectively, $n = 60$ for a ratio of 3:1). The performance of crow O was 93%, 75%, and 90% for ratios of 4, 3, and 2, respectively. The performance of crow G was 80%, 85%, and 87% for ratios of 4, 3, and 2, respectively. As expected, categorization with probe bird vocalizations that had the lowest frequency interval ratio of all probe sounds near the category boundary became increasingly more difficult for the crows. Crow O correctly categorized the probe bird vocalization sounds (70%; binomial test, $p < 0.01$, $n = 40$), whereas crow G showed a tendency but did not reach significance (55%; binomial test, $p = 0.32$, $n = 40$). Overall, however, the crows categorized novel sounds correctly into the appropriate categories, with categorization performance suffering close to the category boundary.

Next, we investigated within-category generalization performance. Within-category generalization predicts that performance is independent from the acoustic details of the FM sound, such as the modulation trajectory and the frequency composition of the sounds. To that aim, we separately analyzed and compared performance to the four probe stimulus classes (linear, logarithmic, quadratic pure-tone FM sweeps, and bird vocalization segments). Both crows showed high performance to all probes containing FM sweeps of different trajectories. (linear: crow O 85%, crow G 85%; logarithmic: crow O 85%, crow G 83%; quadratic: crow O 80%, crow G 85%) (each binomial test, $p < 0.001$, $n = 40$) (Figure 6). As mentioned above, the bird vocalization probes that exhibited only mild frequency modulation were close to the category boundary and thus more difficult for the crows. To summarize, for probe sounds with distinct frequency modulation, the crows categorized performance was independent from the type of modulation trajectory.

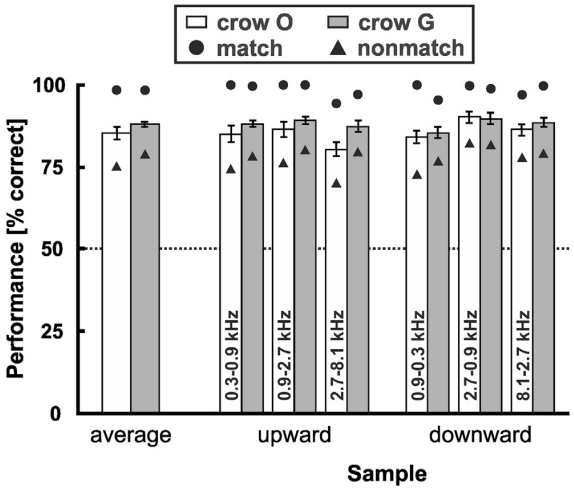

Figure 3. Performance to Familiar Training Stimuli

Both crows responded significantly above chance (dashed horizontal line at 50% performance) to upward and downward FM samples. Columns represent mean performance values averaged across match and non-match trials (error bars: standard error of the mean), circle and triangle symbols reflect mean performance for match and non-match trials separately.

In addition, we investigated whether the frequency range of the 120 pure-tone probe stimuli (linear, logarithmic, and quadratic sweeps) had an influence on behavior. Half of these stimuli had a frequency between 0.3 and 2.7 kHz and were therefore assigned to the group of "low-frequency" stimuli. The other half had a frequency between 0.9 and 8.1 kHz and were grouped as "high-frequency" stimuli. Stimuli including frequencies in the overlapping range of 0.9–2.7 kHz never contained both frequencies lower than 0.9 kHz and higher than 2.7 kHz. The crows performed well above chance regardless of the frequency range of the sample stimuli (each binomial test, $p < 0.001$, $n = 60$) (Figure 6). Crow O responded correctly in 87% and 80% of low frequency and high frequency trials, respectively. Crow G responded correctly in 92% and 77% of low frequency and high frequency trials, respectively. Thus, the crows showed robust within-category generalization irrespective of the frequency range of the probe sounds.

DISCUSSION

Our data show that crows possess categorical auditory working memory. They are able to maintain the FM categories upward and downward in working memory to master an auditory delayed match-to-category task. As a sign of categorical generalization and transfer, the crows instantaneously and without further training matched the remembered novel sample sounds correctly to the upward and downward FM categories, irrespective of other sound parameters. The crows' behavior showed the diagnostic characteristics of categories, namely, sharp category boundaries and within-category generalization: the crows categorically classified the continuous direction of FM into upward and downward while ignoring other sound parameters (such as spectral composition, frequency intervals, or modulation trajectory of the novel sample sounds) within one FM sound category. This suggests that the crows only memorized the direction of the FM, not the other varying sound parameters, when categorizing sounds from working memory.

Auditory Categorization in Birds

Birds have also been shown to discriminate and classify complex sounds. Vocal learners, in particular, rely on acute audition and are known to perceive sounds in a categorical way (Dooling et al., 1995; Burgering et al., 2019). Even pigeons, non-songbirds with an unlearned vocal repertoire, are able to make same/different discriminations across a wide variety of auditory stimuli (Murphy and Cook, 2008; Cook and Brooks, 2009; Cook et al., 2016) and can learn to discriminate among music-derived acoustic elements and sequences (Brooks and Cook, 2010; Hagmann and Cook, 2010; Brooks and Cook, 2010; Cook, 2017). However, previous experiments did not require the birds to flexibly switch between auditory

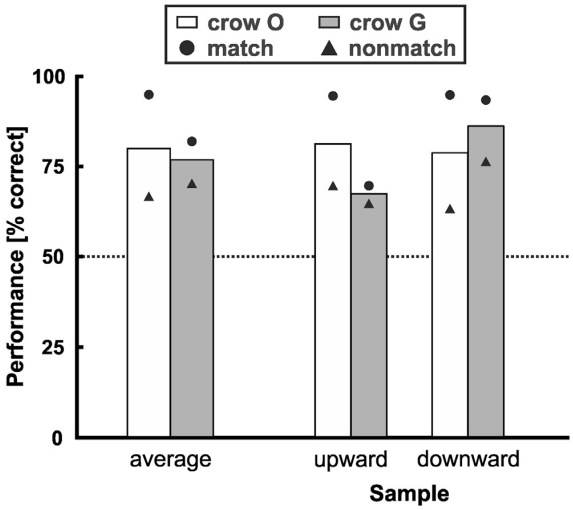

Figure 4. Overall Performance to Novel Probe Stimuli

Both crows responded significantly above chance to upward and downward FM probe samples. Columns represent mean performance values averaged across match and non-match trials (error bars: standard error of the mean), circle and triangle symbols reflect mean performance for match and non-match trials separately.

categories or remember auditory categories in working memory. In these studies, the birds were typically tested in Go/NoGo or forced choice tasks without a delay period. Both temporal and spectral changes in the sounds could be exploited.

Birds are known to categorize complex sounds, such as human speech sounds, based on temporal differences. For instance, budgerigars place vowels /i/, /a/, /e/, and /u/ in phonetically appropriate categories in spite of variation in who is talking and their gender (Dooling and Brown, 1990). When working with synthetic phoneme continua of speech sounds, budgerigars exhibit perceptual phonemic boundaries near the human boundaries for /ba/-/pa/, /da/-/ta/, /ga/-/ka/, /ra/-/la/, and /ba/-/wa/ (Dooling et al., 1995; Dent et al., 1997). Similar perception of speech sound categories has also been shown in quails and zebra finches (Burgering et al., 2019; Kleunder et al., 1987; Ohms et al., 2010). Because the phoneme boundaries rely on temporal differences (or "voice onset time" between the vowel and the consonant), these data suggest that not only sound frequency but also sound timing plays an important role in birds' capability to categorize sounds.

Besides temporal factors, also the spectral composition of sounds can be exploited by birds. In a series of experiments, several songbird species (primarily European starlings) have been shown to perceive pitch relations in a simple tonal melody (Hulse and Cynx, 1985). In particular, songbirds can classify rising as opposed to falling pitch patterns. However, these songbirds preferentially discriminated tonal patterns according to the absolute frequency of the individual element tones in the patterns; they failed to transfer discrimination to a novel frequency range when the training frequency range was shifted. Only when the experimental conditions severely constrained the use of pattern element cues did the songbirds use pitch relations as a secondary strategy (Hulse and Cynx, 1986; Hulse et al., 1984; Braaten et al., 1990). Data like these lead to the conclusion that birds, unlike humans, cannot generalize relative pitch discrimination to new frequencies, thus lacking a conceptual grasp of frequency modulation in complex sounds. However, our data suggest that corvid songbirds can indeed form a conceptual understanding of upward and downward frequency modulation, irrespective of frequency composition.

Auditory Working Memory in Birds

Auditory working memory capabilities have only rarely been studied in birds, mainly because it is difficult to train birds—and nonhuman animals in general—to perform auditory working memory tasks that are similar

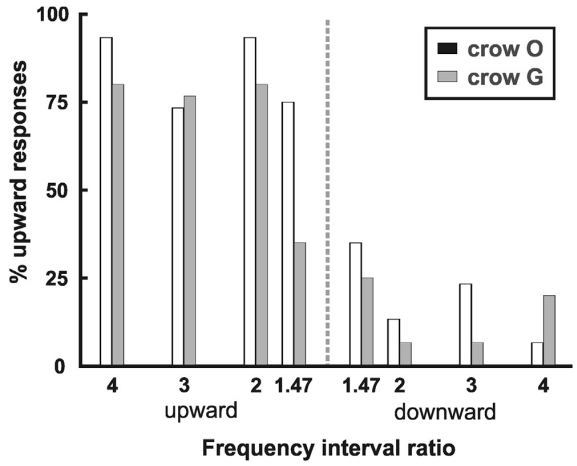

Figure 5. Performance Relative to Category Boundary

Categorization performance to probe stimuli of different frequency interval ratios of upward and downward FM sounds. Performance is depicted as percent correct classification as "upward" category. Vertical dashed line indicates the category boundary at a frequency interval ratio of 1.

to those used in the study of visual memory (Plakke and Romanski, 2016). Nonetheless, a few studies show that European starlings exhibit auditory working memory and show interesting similarities and differences when compared with humans (Zokoll et al., 2007, 2008a, 2008b; Comins and Gentner, 2010). For example, the classical finding of a decay of working memory with increasing delay times in humans and other animals could be reproduced in starlings (Zokoll et al., 2008a, 2008b). In contrast to humans, however, starlings benefited from repeated presentations of sample sounds. Our study adds to these insights by showing that songbirds maintain not only specific sounds in working memory but also overarching auditory categories. Overall, songbirds are therefore valuable models for investigating not only mechanisms of auditory signal processing but also cognitive control functions in the auditory domain.

Categorization of Bird Vocalizations

In contrast to novel pure-tone FM sweeps, novel segments of frequency-modulated bird vocalizations were more difficult to categorize for the crows. One crow reached significant categorization (albeit with less precision than with the pure-tone probes), whereas the other crow showed a tendency but failed significance. Most likely, this difficulty was due to the vocalization segments having the lowest frequency interval ratio of all probe sounds, a ratio that was closest to the category boundary. In addition, the vocalizations were acoustically more complex and richer. Some of them contained broadband noise that potentially could have masked the FMs and additional harmonics that might have distracted the crows. Overall, however, these data suggest that corvids can categorize and remember animal sounds in order to adapt their behavior.

The capability to memorize sound categories may also have adaptive advantages in a world in which objects and events are characterized by multi-modal signals. The semantic grouping of a multitude of unique stimuli into uni-modal categories facilitates the association with stimuli from other sensory modalities that characterize the same members of a class. For instance, social songbirds need to group conspecifics into different categories based on sex, relatedness, or group membership in order to adjust their behavioral responses. Crows recognize group members by identity congruence between visual presentation of a group member and the subsequent playback of a contact call (Kondo et al., 2012). Because corvids can recognize individuals by sound (Wascher et al., 2015) or sight alone (Kondo et al., 2010), the most parsimonious explanation is that they first categorize acoustic and visual stimuli as belonging to an individual and later associate the auditory and visual categories for cross-modal audiovisual recognition of group members. The brain of crows is able to associate stimuli across modality and time (Moll and Nieder, 2015, 2017). However, whether this extends also to more cognitive cross-modal categories remains to be explored.

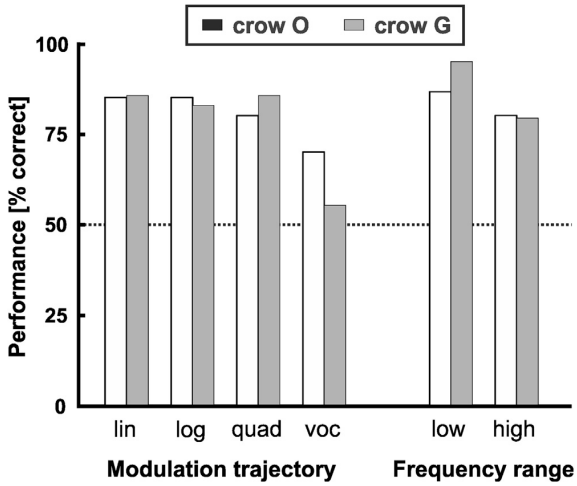

Figure 6. Performance to Probe Stimuli as a Function of Modulation Trajectory and Frequency Range
Chance level is again 50% performance.

Categorization of Pure Auditory Frequency Modulation in Mammals

The categorical discrimination of sounds based on pure frequency modulation has been demonstrated convincingly in a mammal, the Mongolian gerbil (Wetzel et al., 1998; Ohl et al., 2001). In this positive-reinforcement Go/NoGo task, the effects of conditioned fear (CS+) based on FM categories were tested. The gerbils had to change compartments in a shuttle box during ascending FMs (CS+) presentation to avoid foot shock. The gerbils were able to discriminate FM tones by modulation direction and, after familiarization with a number of different FM pairs, transferred the ascending-descending concept to stimuli not heard before (Wetzel et al., 1998). A similar conditioning approach was used in categorization studies with ferrets (Yin et al, 2016, 2020); in one study, individual ferrets were trained to discriminate downward sequences (the target sequence) from upward sequences (the reference sequence), or vice versa (Yin et al., 2010). In both approaches, gerbils and ferrets thus discriminated a fixed FM category stored in long-term memory from deviating sounds.

Although these experiments clearly show perceptual categorization of FMs in gerbils and ferrets, they required the animals neither to flexibly switch between different auditory categories nor to maintain the switching categories in auditory working memory. To address both cognitive aspects, we therefore trained crows on a delayed match-to-category task. This task not only tested the formation of one FM category against other sounds but probed the conceptual flexibility of the crows to switch between rewarded and unrewarded FM categories on a trial-by-trial basis. In addition, the crows could not have succeeded without a working memory for the auditory categories.

Categorical Auditory Working Memory in Monkeys

Categorical auditory perception and working memory have been reported in macaque monkeys. Using a delayed match-to-sample protocol, monkeys were trained to report by an eye movement whether two consecutive human-speech sounds ("dad" versus "bad") or a series of morphed versions of these sounds belonged to the same or different category (Tsunada et al., 2011). The behavioral data showed that monkeys perceived these morphed speech sounds categorically; despite the gradual variation of the acoustic stimulus, the monkeys reliably assigned the morphs to one of the two categories and exhibited a sharp transition boundary between morphed sounds being perceived as dad rather than bad.

Whether the monkeys could also categorize novel morph sounds or other types of speech sounds as a sign of abstract categorization was not tested in this study. We tested this in the current study and found that the crows instantaneously categorized the remembered novel sample sounds correctly to the upward and

downward FM categories, irrespective of other sound parameters. Crows can transfer the semantic grouping criteria they learned to novel and acoustically distinct sounds.

It is worth mentioning that the auditory working memory capacity of monkeys seems to be surprisingly limited and prone to interference. When rhesus monkeys were tested in an auditory delayed match-to-sample task equivalent to the task structure of the current study in which either the first (match condition) or the second test stimulus (nonmatch condition) could be a match and required a response, marked performance differences between the two conditions surfaced. Performance was accurate whenever a match followed the sample directly, but it fell precipitously if (one or two) nonmatch stimuli intervened between sample and match. This drop in accuracy was found to result from an "overwriting" effect, i.e., a retroactive interference from the intervening nonmatch stimulus that was far greater than that observed previously in delayed match-to-sample tasks with visual stimuli. The authors concluded that the monkeys' performance depended on the retention of stimulus traces in the passive form of short-term memory rather than on active working memory (Scott et al., 2012, 2013).

Our data from crows only allow an evaluation of this issue for zero (match condition) or one interfering stimulus (nonmatch condition). The data plotted in Figures 3 and 4 show a similar tendency, namely, a decline in accuracy in the nonmatch condition. Notably, crow G showed only a mild decline in the nonmatch condition when tested with novel probe stimuli (Figure 4). It is also worth mentioning that part (or all) of this performance decline may be due to the crows' bias to respond rather quicker (match condition) to receive a reward earlier. In addition, the performance and response pattern of crows for match and nonmatch conditions is comparable with those we see for visual categorization in delayed match-to-sample tasks (Ditz and Nieder, 2016a, 2016b, 2020; Wagener et al., 2018). Overall, the data suggest that the crows possess active working memory capacities also for auditory stimuli.

Limitations of the Study

This study explored the crows' category generalization capabilities to a limited set of probe stimuli and found that the crows had more difficulty categorizing FM segments of bird vocalizations. One explanation for this finding is that vocalizations showed the smallest frequency interval ratio of all probe stimuli. However, compared with the pure tone training FM sweeps, vocalizations also showed additional harmonics. To demonstrate that crows can generalize FM categories to acoustically richer sounds, the application of multi-harmonic FM sweeps as training and probe stimuli would be helpful. In addition, and to further differentiate active working memory from potential passive short-term memory, the crows' performance when confronted with more than one distractor and for longer delays would be informative. Resistance against distraction over longer delay periods would corroborate the notion of auditory working memory in crows as it is regularly seen in the visual domain.

Resource Availability

Lead Contact

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Materials Availability

This study did not generate new unique Materials.

Data and Code Availability

Original data have been deposited to Mendeley: https://doi.org/10.17632/38x8ktrx7y.1.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101737.

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AUTHOR CONTRIBUTIONS

A.N. and L.W. designed the study, interpreted the data and wrote the manuscript. L.W. performed experiments and analyzed the data. All authors gave final approval for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

Categorical Auditory Working Memory in Crows

Lysann Wagener and Andreas Nieder

Supplemental Information

Categorical auditory working memory in crows

Lysann Wagener and Andreas Nieder

TRANSPARENT METHODS

Subjects

Two 3 years old male carrion crows (*Corvus corone*) were used in this study. The crows were housed in social groups in indoor aviaries. During the training and testing period, the crows were on a controlled feeding protocol. Body weight was measured daily. Food was given as reward during the sessions. Water was *ad libitum* available in the aviary and during the experiments. All procedures were carried out according to the guidelines for animal experimentation and approved by the responsible national authorities, the Regierungspräsidium Tübingen, Germany.

Experimental setup

The birds were placed on a perch in front of a touchscreen monitor (3M Microtouch, 15“, 60 Hz refresh rate) in a darkened operant conditioning chamber (length 1 m, width 0.76 m, height 1 m). One speaker (VISATON B 200 – 6 Ohm) was used to play back the auditory stimuli. The speaker was located 0.6 m in front of the bird and behind the computer monitor. The behavior was controlled by the CORTEX system (National Institute of Mental Health, Maryland, USA) which also stored the behavioral data. An automated feeder delivered either mealworms (*Tenebrio molitor* larvae) or bird seed pellets upon correctly completed trials. An infrared light barrier was installed above the birds' head to which a reflector foil was attached. The crow had to keep its head still within the beam of the light barrier and thereby in front of the touchscreen throughout a trial.

Behavioral task

The crows were trained on a delayed match-to-category task in which they discriminated the direction of upward and downward frequency modulated (FM) sounds (**Fig. 1**). A crow started a trial by positioning its head in front of the monitor whenever a go-stimulus (small white cross) was shown on the screen. Head position was monitored by an infra-red light barrier, and the crows had to maintain the head still throughout the trial. Premature head movements terminated the trial and it was discarded. When the head was in the correct position in front of the monitor, the crows received auditory feedback and the go-stimulus on the screen turned into a white circle for 60 ms. For the further course of the trial the monitor remained black. After a 600 ms silent pre-sample phase, the auditory FM-modulated sample stimulus (300 ms duration) was played. This was followed by a 1000 ms silent delay period during which the crow had to memorize the direction of the frequency modulation (upward or downward) of the sample. In the following test phase, the crow had to match the direction of the FM in the sample to the test stimulus with the same FM direction (i.e. upward to upward FM, and downward to downward FM). If the direction of the FM matched, the crow had to respond by quickly moving its head out of the light barrier to receive a reward.

In 50% of the trials, the first test stimulus (test1) was the matching stimulus ('match condition'). In the other 50% of the trials, the test1-stimlus was a 'non-match' with a FM in the opposite direction of the sample's FM direction ('nonmatch condition'). In this case, the bird had to refrain from responding and wait with a response until the second test stimulus was played which was always a match. Both the test1- and the test2-periods were 900 ms in duration, with the 300 ms test1- and test2-stimuli played right at the beginning of the test-periods (so that the remaining 600 ms of the test-periods were silent). The response interval was shifted by 100 ms due to the inevitably reaction latency relative to physical stimulus onset. Responses to the 'nonmatch stimulus' and no response to either of the two test stimuli were considered as error and also not rewarded. Match and non-match conditions were balanced and pseudo-randomly presented. The crows were first trained with well-known training stimuli. Once the crows reached high performance, we tested if they were able to transfer the upward and downward FM categories to novel stimuli that were occasionally presented among the ongoing discrimination of the training sample stimuli.

Stimuli

A total of 168 auditory frequency modulated stimuli were used in this study. All stimuli had a duration of 300 ms and a 10 ms linear amplitude ramp at the beginning and the end.

Training stimuli. The crows were trained with a fixed set of 6 FM sample stimuli (3 upward and 3 downward sweeps). These ***training sample stimuli*** consisted of linearly rising or falling FM pure tones (**Fig. 2A**). The frequency range of the three upward training sample stimuli were 0.3-0.9 kHz, 0.9-2.7 kHz and 2.7-8.1 kHz. The identical frequency range of the three downward training sample stimuli was 0.9-0.3 kHz, 2.7-0.9 kHz and 8.1-2.7 kHz. Thus, each training sample stimulus had a bandwidth of 1.6 octaves. Each of these sample stimuli had to be matched to its corresponding matching test stimulus. A linearly FM-modulated sweep from 0.3-8.1 kHz was the match for upward FM stimuli, whereas a linear downward sweep from 8.1-0.3 kHz served as a match for downward FM stimuli (**Fig. 2B**).

Probe sample stimuli. Once the crows reliably discriminated and categorized the training stimuli, we tested their ability to transfer the upward and downward FM categories to novel sample sounds (probe stimuli). We tested a total of 80 probe stimulus pairs (each with upward and downward FM modulation) which the crows had never encountered before. Only responses to the first presentation of each unique probe stimulus – before the crows could learn a 'correct' response to these new stimuli - were analyzed. The test-stimuli remained the same as in the training trials.

The probe stimuli were grouped into four classes of FM sweeps: linear, logarithmic and quadratic FM modulation of pure tones, and FM-modulated bird vocalizations. Each of the four classes consisted of 40 unique stimuli (20 upward and 20 downward sweeps). All pure-tone sweeps (including the training, test and probe stimuli) were generated using a custom written MATLAB code. The sounds were saved as wav-files at a sampling frequency of 44.1 kHz.

$$\text{Linear: } f_i(300ms) = f_0 + \beta t, \text{ where } \beta = (f_1 - f_0)/t_1$$

$$\text{Logarithmic: } f_i(300ms) = f_0 * \beta^t, \text{ where } \beta = \left(\frac{f_1}{f_0}\right)^{\frac{1}{t_1}}$$

$$\text{Quadratic: } f_i(300ms) = f_0 + \beta t^2, \text{ where } \beta = (f_1 - f_0)/{t_1}^2$$

The pure-tone probe stimuli differed in frequency-modulation range and frequency content. The frequency-modulation ranges was quantified by the frequency interval ratio, which is the maximum frequency contained in the FM sound divided by the minimum frequency ($f_{max} : f_{min}$). The probe FM sweeps had frequency interval rations of 2:1 (1 octave), 3:1 (1.6 octaves; **Fig. 2C**) and 4:1 (2 octaves).

The frequency content was roughly divided into 'low' and 'high' frequencies. The 'low frequency' probe stimuli covered frequencies between 0.3-2.7 kHz (examples shown in **Fig. 2D**), whereas the 'high frequency' stimuli covered 0.9-8.1 kHz. Stimuli including frequencies in the overlapping range of 0.9 to 2.7 kHz were never both, lower than 0.9 kHz and higher than 2.7 kHz at once. Likewise, none of the stimuli laid exclusively within the overlap, so that each stimulus could be related to 'low' or 'high' based on whether it reached into the range of 0.3-0.9 kHz or 2.7-8.1 kHz, respectively.

The bird vocalization probe stimuli were excerpts of bird vocalizations (for example, *Parus major*, *Sturnus vulgaris*, *Buteo buteo*, *Alcedo atthis*) (downloaded from http://www.xeno-canto.org/) which have been recorded at 16-bit resolution and almost all a sampling rate of 44.1 kHz (except for two at 48 kHz and one at 16 kHz). These were further modified using Adobe Audition 3.0 and Audacity 1.0.0. From all vocalizations, a 300 ms segment covering a monotonic frequency change was extracted. The amplitude of the signal was equalized to the pure-tone stimuli and 10 ms ramps were added. Each vocalization probe stimulus was used

with its original FM-sweep direction (8/20 upward, 12/20 downward) for one FM category, and as a temporally inverted version for the other FM category. The average frequency interval ratio of the vocalization probe stimuli was 1.47:1 (± 0.25 STD).

Transfer to novel FM stimuli was tested during 10 sessions. In each session we used four different stimuli per probe class (linear, logarithmic, quadratic and bird vocalization sweeps) with two upward and two downward sweeps per class (or two probe stimulus pairs per probe class). The upward and downward sweep of each probe pair covered exactly the same frequency range. The pure-tone probe stimuli for each daily session were selected so that each session contained 2 'low frequency' and 2 'high frequency' linear, logarithmic and quadratic sweeps. For the first 5 sessions of the experiment, only pure-tone probe stimuli with a bandwidth of 1.6 octaves were used, whereas for the second 5 sessions stimuli with 1 and 2 octaves were used (6 of each in each session).

Each session consisted of an average of 577 completed pseudo-randomized trials for crow O and 566 completed trials for crow G. Of those, the familiar training sample stimuli were presented in 88% of the trials and probe sample stimuli were presented pseudo-randomly in the other 12% of the trails. A small proportion of probe stimuli prevented the crows to learn response patterns for those stimuli. Familiar training sample stimuli as well as probe sample stimuli were always followed by the same familiar test stimuli also used for training (see 'Training stimuli'). In either case, the crows were rewarded for every correct response to a match to promote category maintenance. Only responses to the first presentation of each unique probe stimulus were analyzed. During this first presentation of the probe stimulus, the crows were not able to learn a 'correct' response but had to infer category membership based on their previous knowledge acquired with training stimuli.

Data analysis

The percent correct responses, i.e. the number of correct trials divided by the total number of completed trials, was calculated as a measure of behavioral performance. Performance was calculated separately for up- and downward sweeping training sample stimuli and the classes of probe stimuli. To assess transfer of upward and downward FM categories, only the first trial for each unique probe FM stimulus was included. Probe trial performance therefore quantified the percentage of correctly answered first probe stimuli. This ensured that the crows could not learn how to respond to probe trials but relied on transferring their categorical perception.

Error types: The only type of error possible in the match condition is a type2-error (crow does not respond to match) because the trial ends after presentation of test1 (match). In the nonmatch condition, the crows only made type1-errors (false alarms; crow responds to nonmatch) because the crows always responded to either test1 or test2 (with the exception of a single trial across all sessions). The percent correct performance for match and nonmatch conditions separately therefore indicate all possible types of errors the crows made.

Study 2: Crows protect visual working memory against interference

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SHORT COMMUNICATION

Crows protect visual working memory against interference

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ABSTRACT

Working memory, the ability to actively maintain and manipulate information across time, is key to intelligent behavior. Because of the limited capacity of working memory, relevant information needs to be protected against distracting representations. Whether birds can resist distractors and safeguard memorized relevant information is unclear. We trained carrion crows in a delayed match-to-sample task to memorize an image while resisting other, interfering stimuli. We found that the repetition of the sample stimulus during the memory delay improved performance accuracy and accelerated reaction time relative to a reference condition with a neutral interfering stimulus. In contrast, the presentation of the image that constituted the subsequent non-match test stimulus mildly weakened performance. However, the crows' robust performance in this most demanding distractor condition indicates that sample information was actively protected from being overwritten by the distractor. These data show that crows can cognitively control and safeguard behaviorally relevant working memory contents.

KEY WORDS: Corvid songbird, Visual working memory, Distractor resistance

INTRODUCTION

The maintenance of information over brief delay periods can be achieved by different cognitive systems (Shevlin, 2020). In the case of simple short-term memory (such as iconic and echoic memory), a stimulus trace is temporarily retained in a passive, implicit way; short-term memory is fragile and highly susceptible to erasing by a successive stimulus. In contrast, working memory addresses a system by which the memory contents depend on attention and can be held and manipulated towards a goal in an active, explicit state; for as long as attention is directed at memorized relevant information, it can be protected not only from passive decline but also from interfering irrelevant stimuli (Luck and Vogel, 1997; Cowan, 2008; Baddeley, 2012; Carruthers, 2013; Oberauer, 2019; Nieder, 2022).

When exploring memory capacities in animals, this distinction is crucial. Animals are typically tested in variations of 'delayed response tasks' that contain a brief temporal gap between a stimulus and a response. However, an animal's success in a delayed response task does not yet indicate working memory because passive short-term memory typically suffices to explain performance (Nieder, 2022). One way to segregate passive short-term memory from active working memory is the presentation of interfering stimuli during memory retention. With only passive short-term memory at work, memory performance suffers greatly after distraction (Scott et al., 2012). However, with working memory capabilities, animals are able to largely ignore and filter out distracting information (Jacob and Nieder, 2014). Of course, animals – and corvids in particular – can store information for much longer durations in long-term memory (Kamil et al., 1994; Balda and Kamil, 1989; Olson, 1991; Olson et al., 1995; Gould-Beierle, 2000); however, to access this information from long-term memory, it needs to be retrieved into working memory.

Several bird species have been tested successfully for their ability to memorize information across short temporal gaps (e.g. Blough, 1959; Roberts, 1980; Regolin et al., 2005; Veit et al., 2015; Rinnert et al., 2019; Rinnert and Nieder, 2021). The delayed match-to-sample (DMS) task is a suitable task to investigate memory capacities in animals (Hunter, 1913; Lind et al., 2015). In the DMS task, an animal is first presented with a sample stimulus that is afterwards removed. After a delay period in which no stimulus is displayed, two or more choice stimuli are presented. The subject receives a reward for selecting the one that matches the sample. Different species of birds, such as pigeons (Blough, 1959; Roberts, 1980; Johnston et al., 2019), chickens (Nakagawa et al., 2004), black-capped chickadees, dark-eyed juncos (Brodbeck and Shettleworth, 1995), jays (Olson et al., 1995) and carrion crows (Goto and Watanabe, 2009; Veit et al., 2014; Hartmann et al., 2018; Ditz and Nieder, 2016; 2020; Wagener and Nieder, 2017; 2020; Balakhonov and Rose, 2017) can master the DMS task. However, so far it is not known whether corvids or other birds can actively protect memorized information against interference as an essential feature of working memory. In the current study, we therefore modified the classic DMS task by introducing an interfering stimulus following the presentation of the sample halfway through the delay period (Fig. 1). To succeed in the face of distraction, the animals need to actively maintain relevant sample information and to safeguard it by filtering out distractors (Jacob and Nieder, 2014; Jacob et al., 2018).

We considered two hypotheses. Target representation in memory could deteriorate in the face of strong task-irrelevant distractors, indicating that crows rely primarily on interference-vulnerable and passive short-term memory. Alternatively, the crows' memory performance could remain largely unaffected by interfering information, suggesting active filtering and suppression of distractor information characteristic of explicit cognitive control of memory contents. We found clear evidence for the latter.

MATERIALS AND METHODS

Subjects

One 2 year old female and one 2 year old male carrion crow (*Corvus corone* Linnaeus 1758) were used in this study. The crows were housed in social groups in indoor aviaries. During the training and

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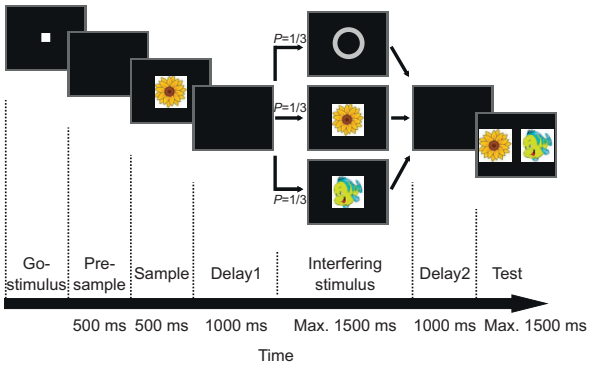

Fig. 1. Schematic illustration of the delayed match-to-sample task with interfering stimuli. In each trial sequence (left to right), one of three interfering stimuli was shown halfway through the delay period: a neutral gray circle, repetition of the sample stimulus, or an image that served as the non-match in the impending test period. The crows needed to peck at the interfering stimuli to continue the trial. The crows indicated their choice in the test period by pecking at the selected test image (here, the flower would be the correct match).

testing period, they were on a controlled feeding protocol. Food was given as a reward during the sessions. Water was available *ad libitum* in the aviary and during the experiments. All procedures were carried out according to the guidelines for animal experimentation and approved by the responsible national authorities, the Regierungspräsidium Tübingen, Germany.

Experimental setup

The crows were placed on a perch in front of a touchscreen monitor (3M Microtouch, 15 inch, 60 Hz refresh rate) in a darkened operant conditioning chamber (length 1 m, width 0.76 m, height 1 m). The behavior was controlled by the CORTEX system (National Institute of Mental Health, Bethesda, MD, USA) which also stored the behavioral data. An automated feeder delivered either mealworms (*Tenebrio molitor* larvae) or bird seed pellets upon correctly completed trials. An infrared light barrier was installed above the crows' head to which a reflector foil was attached. Except for the distractor and test periods, the crow had to keep its head still within the beam of the light barrier and thereby in front of the touchscreen throughout a trial.

Behavioral task

The crows were trained on a DMS task in which they matched images (Fig. 1). A crow started a trial by positioning its head in front of the monitor whenever a go-stimulus (a small white square) was shown on the screen. When the head was in the correct position in front of the monitor, the crows received auditory feedback. After a 500 ms pre-sample phase with no stimulus, the sample stimulus (500 ms duration) was displayed. Colorful complex images were used as stimuli.

The sample was followed by a 1000 ms delay period (delay1) with a blank screen. Next, one of three interfering stimuli was shown in equal trial proportions (one-third) and pseudo-randomly interleaved: a gray circle that was never shown in the sample or test periods (neutral-image trials), the initially shown sample image (repeat-sample trials) or the image that was shown as a non-match stimulus in the subsequent test period (distractor trials). To ensure that the crow was perceiving the interfering stimulus, it had to peck at it within 1500 ms to continue the trial, and thereafter to resume the correct head position in front of the monitor. After a second 1000 ms delay period (delay2), the test period displayed two choice images side by side. To receive a reward, the crow had to peck at the test stimulus that matched the sample ('match') within 1500 ms while ignoring the non-matching stimulus ('non-match'). Match and non-match were pseudo-randomly and equally often shown on the left or right side. For every session, three new sample and non-match images were selected. Responses to the non-match were considered as error and not rewarded. Premature head movements (except during the interfering stimulus and test period) ended the trial, which was then discarded. The tests began once the crows' accuracy reached at least 75% correct responses per session. Each session consisted of an average of 412 completed trials for crow 1 and 446 completed trials for crow 2.

Data analysis

The percentage of correct responses, i.e. the number of correct trials divided by the total number of completed trials, was calculated as a measure of behavioral accuracy. As a second measure, the reaction time (RT), i.e. how quickly the crows pecked the correct match stimulus in the test phase, was calculated. Accuracy and RT were calculated separately for the three interfering stimulus conditions. The relationship between accuracy and RT was measured using Pearson correlation. MATLAB (version R2020b, MathWorks Inc., Natick, MA, USA) was used for all data analyses.

RESULTS AND DISCUSSION

The two crows performed a modified version of a visual DMS task, in which a task-irrelevant, interfering image was presented halfway through the working memory period (Fig. 1). Sample and test images varied in individual trials so that the crows had to flexibly memorize what they saw on a trial-by-trial basis. To test the crows' working memory, one of three different types of 'interfering stimuli' was presented within the delay period: 'neutral-image trials' as a reference condition, 'repeat-sample trials' and 'distractor trials'. The crows' performance in these three conditions was compared using percentage correct performance (performance accuracy) and RTs as quantitative parameters.

Across all sessions, crow 1 reached a mean (±s.d.) performance accuracy of 88.9±4.5% (across 50 sessions), while crow 2 showed a mean accuracy of 90.4±3.8% (across 63 sessions) (Fig. 2A,D). The average accuracy of both crows with all three trial types in each daily session was significantly above the 50% chance level (each crow $P<0.001$, two-tailed binomial test). The crows' accuracy systematically differed between the three interfering stimulus conditions (each crow $P<0.001$, ANOVA) (Fig. 2B,E). Relative to the reference accuracy for the neutral-image trials (crow 1: 90.8±4.6%; crow 2: 92.8±3.3%), accuracy increased for repeat-sample trials (crow 1: 98.0±2.6%; crow 2: 96.4±2.5%; each crow $P<0.001$; paired-sample *t*-test, Bonferroni corrected) (Fig. 2B,E). In contrast, accuracy decreased relative to the neutral-image trials for both crows in distractor trials (crow 1: 80.2%±7.8%; crow 2: 83.6±7.0%; each crow $P<0.001$; paired-sample *t*-test, Bonferroni corrected) (Fig. 2B,E). Thus, across both crows, repetition of the sample during the memory delay enhanced accuracy on average by 5.25%, while the presentation of the non-match stimulus deteriorated accuracy by 9.9%.

As second performance parameter, we explored RT. Across all sessions, crow 1 had a mean (±s.d.) RT of 516.0±29.7 ms (across 50 sessions), while crow 2 showed a mean RT of 549.3±69.9 ms (across 63 sessions). The crows' RT systematically differed between the three interfering stimulus conditions (each crow $P<0.001$,

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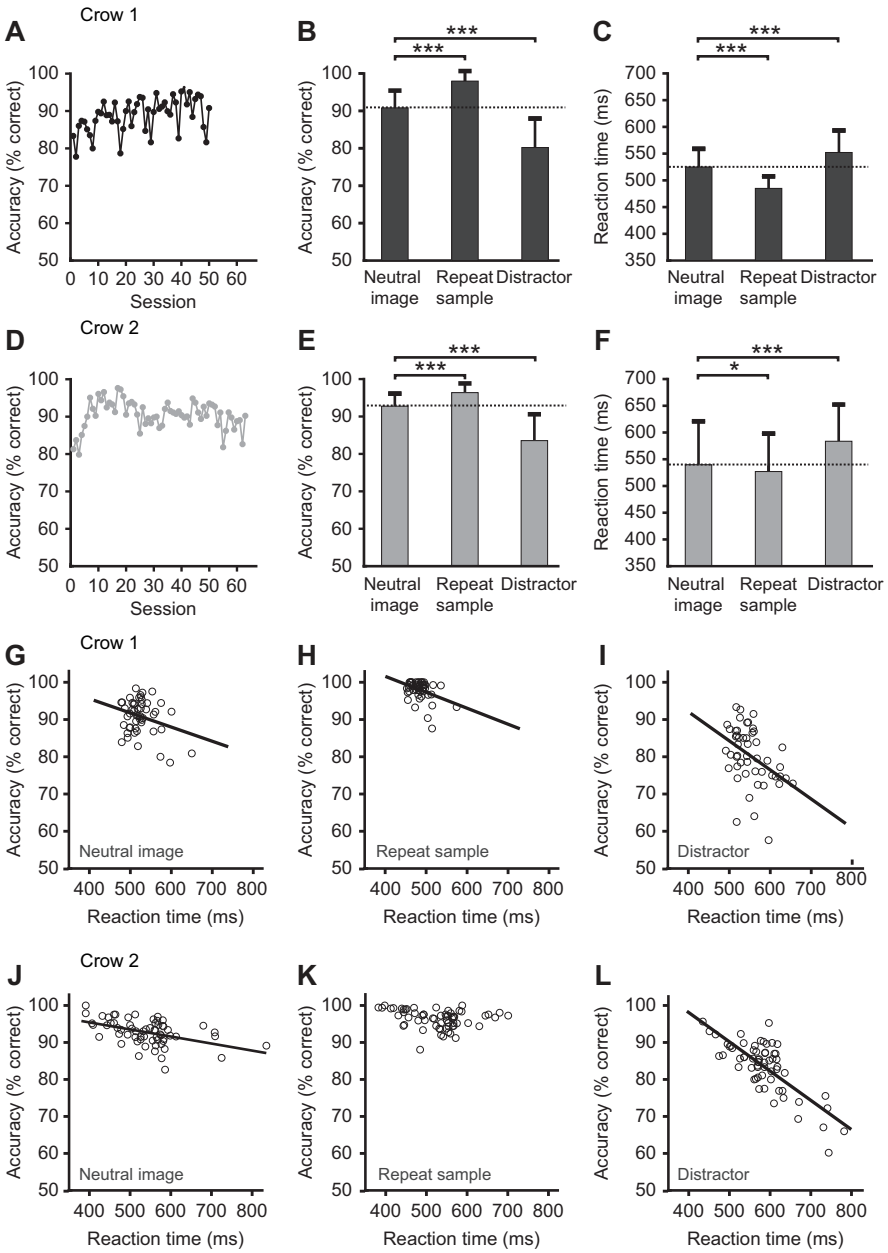

Fig. 2. Behavioral data. (A) Accuracy (mean values) of crow 1 across sessions. (B) Average accuracy of crow 1 for the neutral, repeat-sample and distractor trials. Shown are the means across sessions and the s.d. The dotted line indicates the reference value for neutral trials. (C) Average reaction time of crow 1 for the neutral, repeat-sample, and distractor trials. Shown are the means across sessions and the s.d. The dotted line indicates the reference value for neutral trials. (D) Accuracy of crow 2 across sessions. (E) Average accuracy of crow 2 for the neutral, repeat-sample and distractor trials. Same layout as in B. (F) Average reaction time of crow 2 for the neutral, repeat-sample and distractor trials. Same layout as in C. (G–I) Correlation of accuracy with reaction time for neutral trials (G), repeat-sample trials (H) and distractor trials (I) across all sessions for crow 1. (J–L) Same as in G–I, but for crow 2.

ANOVA) (Fig. 2C,F). Relative to the reference RT for the neutral-image trials (crow 1: 525.0±33.9 ms; crow 2: 539.7 ±81.3 ms), RT decreased for repeat-sample trials (crow 1: 485.0 ±22.5 ms; crow 2: 527.0±70.9 ms; crow 1: $P<0.001$, crow 2: $P<0.029$; paired-sample t-test, Bonferroni corrected) (Fig. 2C,F). In contrast, RT increased relative to the neutral-image trials for both crows in distractor trials (crow 1: 552.1 ms±41.2 ms; crow 2: 583.7 ±68.6 ms; each crow $P<0.001$; paired-sample t-test, Bonferroni corrected) (Fig. 2C,F). Thus, repetition of the sample during the memory delay sped responses up by 26.4 ms on average, while presentation of the non-match stimulus slowed responses down by 35.6 ms across both crows.

The findings so far indicated an inverse relationship between accuracy and RT: more difficult conditions resulted in longer RTs. To systematically explore this relationship, we correlated accuracy and RT on a session-by-session basis. For each crow individually, we found a significant negative correlation of accuracy with RT (crow 1: $r=-0.377$, $P=0.007$; crow 2: $r=-0.731$, $P<0.001$; Pearson correlation). We tested this correlation for each of the three trial conditions and two crows separately. Significant negative correlations were found in crow 1 for all three conditions (neutral-image trials: $r=-0.281$, $P=0.048$; repeat-sample trials: $r=-0.377$, $P=0.007$; distractor trials: $r=-0.415$, $P=0.003$; $n=50$) (Fig. 2G–I). Similarly, significant negative correlations were found in crow 2 for

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neutral-image trials (r=−0.463, P<0.001; n=63) and distractor trials (r=−0.771, P<0.001) (Fig. 2J,L), whereas repeat-sample trials showed only a negative tendency (r=−0.239, P=0.061) (Fig. 2K). This confirms that performance accuracy and RT were negatively correlated irrespective of the interfering stimulus condition.

Our data show that the crows were affected by different types of interfering information during the delay period. Importantly, the crows managed surprisingly well to safeguard the relevant sample stimulus from demanding distraction. The results indicate that crows can actively protect relevant sample information from being erased by the distractor, thus emphasizing the crows' cognitive aptitude (Nieder, 2017). Crows possess active working memory, enabling them to cognitively control the memorization of relevant information. Whether interfering information during delay times longer than the 3 s used in the current study would elicit comparable effects remains to be seen.

To guarantee that the interfering stimuli were perceived, we required the crows to peck at them. This constraint prevented us from testing performance without any interfering stimulus as a reference situation, as it would have lacked a motor response that alone could explain potential differentiating effects compared with interfering stimulus conditions. Instead, we used the neutral-image condition with a simple circle as a performance reference. In preceding pilot tests, a circle as the interfering stimulus was found to elicit indifferent accuracy and RT performance compared with no interfering stimulus in crow 1.

Relative to the neutral-image condition, significant improvement in performance (in terms of both accuracy and RT) was found for the repeat-sample trials in both crows. This finding suggests that the crows benefitted from repeating the relevant sample information in working memory to achieve higher performance. Such maintenance of relevant information by repetition in working memory is captured in Baddeley's working memory model (Baddeley, 2003).

Relative to the neutral-image condition, a mild but significant decay in performance (in terms of both accuracy and RT) was found for the distractor trials with the non-match stimulus as an interfering stimulus in both crows. Distractor trials were certainly the most difficult condition because not only did the non-match stimulus belong to the same complex picture category as the match, a situation known to elicited the highest distraction (Yoon et al., 2006; Sreenivasan and Jha, 2007), but also the distractor was the only other response option besides the match in the test phase. The crows' continued high performance in this distractor condition clearly indicates that sample information was actively protected and cognitively controlled from being overwritten by the distractor. It seems crows can attenuate the processing of distracting information due to endogenous attentional biasing towards relevant sample information during working memory maintenance (Quest et al., 2022). At the same time, more frequent selection of the distractor in the test phase (and thus more errors) also signifies that the distractor was not eliminated but held in memory. These findings suggest that crows can maintain more than one item at a time in working memory. This has also been suggested for visual change detection tasks in humans, pigeons and crows (Gibson et al., 2011; Balakhonov and Rose, 2017).

In both crows and across interfering stimulus conditions, performance accuracy and RTs were negatively correlated. Thus, higher RTs were associated with higher error rates. That trials with longer RTs are more likely to be errors has also been widely reported for perceptual decision making in humans and other primates when task difficulty is fixed (Carter et al., 1998; Shevinsky and Reinagel, 2019). Only rats show a positive correlation (Shevinsky and Reinagel, 2019). In that respect, crows tend toward producing a more primate-like behavioral pattern.

Why are interfering stimuli not entirely suppressed or filtered out? In the ecological environment of an animal, any stimulus could potentially contain relevant information, maybe even more important information than the task at hand (Berti and Schröger, 2003). It would therefore be maladaptive to completely ignore interfering stimuli. The 'supervisory attentional system' has to react to unexpected and potentially meaningful stimuli to be adaptive (Norman and Shallice, 1986). Working memory is able to coordinate the maintenance of distractibility and the focus on the task at hand; the more difficult and attention-demanding a memory task, the less distraction is seen (Berti and Schröger, 2003).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: L.V., A.N.; Methodology: L.W., P.R., A.N.; Software: L.W., L.V.; Validation: L.W., L.V., A.N.; Formal analysis: L.W., P.R.; Investigation: L.W., P.R., L.V.; Data curation: A.N.; Writing - original draft: L.W., A.N.; Visualization: L.W., A.N.; Supervision: A.N.; Funding acquisition: A.N.

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Data availability
All relevant data can be found within the article.

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Study 3: Comparing the face inversion effect in crows and humans

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ORIGINAL PAPER

Comparing the face inversion effect in crows and humans

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Abstract Humans show impaired recognition of faces that are presented upside down, a phenomenon termed face inversion effect, which is thought to reflect the special relevance of faces for humans. Here, we investigated whether a phylogenetically distantly related avian species, the carrion crow, with similar socio-cognitive abilities to human and non-human primates, exhibits a face inversion effect. In a delayed matching-to-sample task, two crows had to differentiate profiles of crow faces as well as matched controls, presented both upright and inverted. Because crows can discriminate humans based on their faces, we also assessed the face inversion effect using human faces. Both crows performed better with crow faces than with human faces and performed worse when responding to inverted pictures in general compared to upright pictures. However, neither of the crows showed a face inversion effect. For comparative reasons, the tests were repeated with human subjects. As expected, humans showed a face-specific inversion effect. Therefore, we did not find any evidence that crows—like humans—process faces as a special visual stimulus. Instead, individual recognition in crows may be based on cues other than a conspecific's facial profile, such as their body, or on processing of local features rather than holistic processing.

Keywords Face inversion · Corvid · Categorization · Delayed matching-to-sample · Social cognition

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Introduction

Compared to stimuli of other categories, in humans, recognition and memory of faces is disproportionately impaired when faces are presented upside down even though both upright and inverted stimuli carry the same physical information (Yin 1969; Rhodes et al. 1993; Rossion 2009). While inversion reduces recognition of non-face stimuli by only 10%, recognition of faces is reduced by about 25% (Carey and Diamond 1977; Diamond and Carey 1986). This 'face inversion effect' has been interpreted as an indicator for specialised or proficient processing of faces compared to other stimuli (Liu and Chaudhuri 2003), possibly reflecting a different mechanism (Farah et al. 1995).

Inversion of a stimulus impedes the configural processing of this stimulus (Towler and Eimer 2016), i.e., the encoding of spatial relations between different features, such as the distance between the eyes. Our ability to recognise faces is thought to rely on such configural (or holistic) processing (Bartlett and Searcy 1993; Rhodes et al. 1993; Collishaw and Hole 2000; Maurer et al. 2002). This configural processing appears early in life (Turati et al. 2004; Simion and Giorgio 2015) and seems to mature with time (de Heering et al. 2007; Cassia et al. 2009). Hence, some researchers argue that this domain-specific processing of faces is innate (Farah et al. 1995). However, others suggest it is also the result of our experience and thus reflects expertise for processing faces (Diamond and Carey 1986; Gauthier and Tarr 1997). This expertise is achieved by exploiting, where possible, a configural assembly of an object's features, and can thus be achieved with any type of stimulus where individual stimuli share many similar features (Gauthier and Tarr 1997; Gauthier et al. 1998). Diamond and Carey (1986), for example, report that 'dog experts' show an inversion effect for dog pictures. Furthermore, certain non-face stimuli are

sensitive to inversion, for example words or body postures (Reed et al. 2003). Thus, the face inversion effect is not necessarily confined to conspecific's faces. Rather than reflecting a domain-specific process of face perception, the effect could be a result of expertise. Taken together, it has been argued that a specialised processing of faces might be due to an innate predisposition that matures with exposure (Simion and Giorgio 2015).

Arguably, humans process faces in a specialised manner because faces represent highly relevant cues offering a range of information about, for example, identity, age, sex, or emotional states of social partners (Todorov et al. 2008; Leopold and Rhodes 2010). However, humans are not the only animals that need to differentiate between individuals (Rosa Salva et al. 2015): the face inversion effect as an indicator for specialised face processing has also been investigated in non-human animals. Chimpanzees seem to exhibit a face inversion effect (Parr et al. 1998; Parr 2011a; Dahl et al. 2013), whereas research with rhesus monkeys reports more mixed results (Parr et al. 1999; Parr 2011b). This inconsistency has been attributed to the use of unsuitable methods (Dahl et al. 2013). Aside from primates, so far only a handful of other non-human species have been investigated. Socially living sheep, for example, are able to differentiate between faces of their conspecifics (Tate et al. 2006) and also show a face inversion effect (Kendrick et al. 1996), whereas pigeons do not (Phelps and Roberts 1994).

In the present study, the face inversion effect was investigated in crows. There are two reasons why corvids are an interesting model for studying the face inversion effect. First, corvids, similarly to humans and great apes, show a range of socio-cognitive abilities (e.g., Ostojić et al. 2013; Clayton and Emery 2015; Legg et al. 2015) that require them to differentiate between individuals in diverse contexts. For example, they might need to distinguish between different observers when protecting their caches from them—indeed, scrub-jays and ravens have been found to keep track of which individuals do and do not know about their caches and thus do or do not pose a threat to their caches (Dally et al. 2006; Bugnyar 2011). Furthermore, ravens are known to be aware of relationships between members of their social group (Massen et al. 2014) and adjust their willingness to cooperate with a partner based on identity (Massen et al. 2015). Thus, corvids seem to attend to the identity of their social partners both in cooperative and in competitive situations. Second, previous work suggests that corvids can recognise individuals (Kondo et al. 2012) and are also able to recognise conspecifics using visual cues alone: Rooks can differentiate between their partner and other conspecifics shown on video (Bird and Emery 2008), and carrion crows can be trained to differentiate between full-body pictures of conspecifics (Braun 2013). Hence, the ability to recognise conspecifics and the relevance of the identity of different conspecifics suggests that for corvids, conspecifics represent a relevant stimulus. Consequently, we aimed to assess a potential face inversion effect for conspecific faces as an indicator of specialised processing of faces.

Given the repeated exposure of captive crows to human faces, crows might have developed an expertise for human faces, similarly to humans who developed an expertise for dogs (Diamond and Carey 1986). Hence, our second aim was to investigate whether another stimulus of everyday relevance for captured crows could elicit a face inversion effect: the human face. Previous research supports this prediction because both hand-raised (von Bayern and Emery 2009) and wild corvids (Marzluff et al. 2010; Clucas et al. 2013) have been found to attend to human faces. Furthermore, American crows recognise humans based on their face more than 2 years after the initial presentation (Marzluff et al. 2010) and can differentiate between male and female human faces from coloured pictures (Bogale et al. 2011). Thus, it is likely that crows can use facial cues to differentiate between humans.

To test the hypothesis that birds of the crow family show performance disruption when recognising inverted compared to upright faces, we administered a delayed matching-to-sample task to carrion crows in Experiment 1. Specifically, we compared performance when crows had to recognise: (1) crow faces and non-face control stimuli (side view of a fish), both inverted and upright and (2) human faces and non-face control stimuli (interior of a house). Non-face controls were chosen based on their similarity to the human/crow face stimuli. If faces are 'special' for crows, they should have an impaired performance for inverted images compared to upright images. This impaired performance should further be more pronounced when responding to faces compared to when responding to non-face stimuli. In Experiment 2, we compared the crows' performance to that of human participants using the same stimuli and setup.

Materials and methods

A possible face inversion effect was investigated in a delayed matching-to-sample task. Two crows and 20 human participants were tested. In the following, we outline the procedures and setup used for both crows (Experiment 1) and humans (Experiment 2).

Investigating a face inversion effect in carrion crows (Experiment 1)

Subjects and housing

Two male carrion crows, aged 3 years (Walt) and 2 years (Hugo), participated in the experiment. The crows were

housed in large indoor aviaries (360 × 240 cm × 300 cm) side by side in groups of four at the Animal Physiology lab, University of Tübingen, Germany. The crows had been taken from the institute's breeding stock (Hoffmann et al. 2011). The birds were kept on a controlled feeding protocol for the duration of the experiment and earned food during and, if necessary, after the daily tests. Body weight was measured daily. Outside of testing, the birds' diet consisted of chick meat and mashed birdseeds. Water was provided ad libitum in the aviary and during testing. Training and data collection lasted from July to October 2016. All experimental procedures were approved by the local ethical committee and authorised by the national authorities (Regierungspräsidium Tübingen).

General procedure

The birds were trained and tested on the matching-to-sample task in a darkened operant conditioning chamber (Fig. 1a). The CORTEX program (National Institute of Mental Health, MD, USA) was used for stimulus presentation and measuring the birds' performance as error rates. Visual stimuli were displayed on a touch screen monitor (ART development PS-150, 15'', 60-Hz refresh rate), allowing the birds to respond by pecking at stimuli shown on the screen. Leather jesses secured birds loosely to their perch.

Rewards (Beo Special pearls or mealworm beetle larva) for 75% of correct trials were delivered with an automated feeder below the screen. Additionally, birds received auditory feedback with specific tones for correct and incorrect trials. Birds could initiate a trial by placing their head in an infra-red light barrier: in combination with a reflector foil attached to the birds' head the light barrier was activated when the birds were positioned in front of the screen and facing it. Trials were aborted and not counted when the crow left the light barrier during sample presentation. The retainer of the reflector of the light barrier was implanted under general anaesthesia onto the birds' skull for experiments conducted prior to the present study. For a description of surgical procedures, see, e.g., Veit and Nieder (2013). A Go-stimulus (a small white square) was presented on the screen to indicate a new trial (Fig. 1b). A short click indicated the activation of the light barrier and the Go-stimulus disappeared (pre-sample phase). Next, the birds saw a sample stimulus at the centre of the screen (i.e., one of the images described below). After a short delay, two test stimuli, the match and the non-match stimuli, were shown left and right of the centre. The birds had to respond within 3000 ms by pecking one of the stimuli. During training, the delay between sample and test stimuli as well as time-out after incorrect responses were adjusted depending on performance.

In case of an incorrect response, the particular trial was presented in a delayed and pseudo-randomized way until all stimuli combinations were shown once. Only during training, but not during data collection, the retry occasionally took place immediately after an incorrect trial. This was done when birds started to develop a side bias or when performance dropped to chance level once a new stimulus type was introduced.

Birds received between 300 and 480 correct trials a day during training.

Material

The pictures used had been downloaded from google images and flickr.com. Pictures of human faces were selected with permission from the face database provided by the Max Planck Institute of Biological Cybernetics in Tübingen, Germany (Troje and Bülthoff 1996). Pictures of all stimuli were achromatic and brightness was equalised. Pictures were between 45 × 43 and 77 × 47 pixels in size. When performing the tasks, the distance between the birds' eyes and the screen was around 7 cm (Walt) and 9 cm (Hugo), creating an angular diameter of 17.1 and 16.3, respectively.

For data collection, four different categories of stimuli were used (Fig. 2): profiles of crow faces, human faces, house interiors, and fish. The pictures of the crow profile

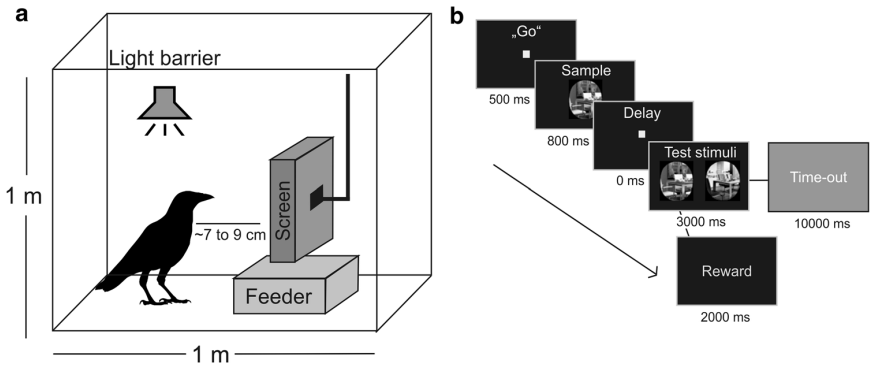

Fig. 1 **a** Set-up for Experiment 1. Crows sat in an operant conditioning chamber measuring 100 × 76 × 100 cm. During testing, the doors of the chamber were kept closed to minimise disruption and to avoid reflections on the screen. **b** Delayed matching-to-sample task used in Experiment 1 and 2. Presentation times varied depending on training progress

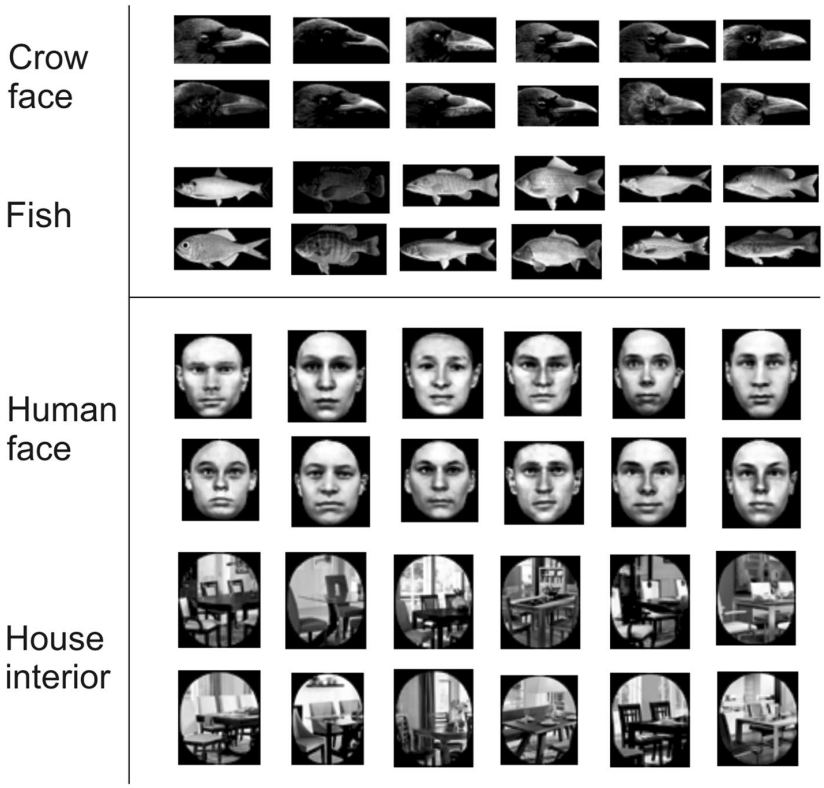

Fig. 2 Stimuli used for testing. Crows were tested on four categories of stimuli: crow faces and corresponding controls (i.e., fish), and human faces and corresponding controls (i.e., house interior)

were from different individuals and of the fish from different species of fish. The crows were not familiar with the crows depicted. There is some indication that for jungle crows the shape of the beak might be used to discriminate between individuals (Kondo and Izawa 2014). Consequently, due to the loss of information about beak size and shape when viewed frontally, the profile might be relevant when recognising conspecifics. Indeed, previous research showed that birds recognise faces in full or ¾ profiles (Trillmich 1976; Brown and Dooling 1992). Moreover, crows rarely see a frontal view of conspecific faces due to their visual scanning behaviour (Fernández-Juricic et al. 2010). Hence, in the present study profiles of carrion crows' heads were used rather than their faces. Note that using the profile was also a practical decision: it was not feasible to acquire a range of portraits of crows facing straight forward. One reason for this might be that corvids exhibit a lot of head movements to scan their environment (Fernández-Juricic et al. 2010) and thus rarely look straight into a camera.

Pictures of fish served as non-face controls for the crow faces and pictures of house interiors as non-face controls for the human faces. Fish were used as non-face controls for two reasons: first, pictures of different fish species were readily available in the same orientation (profile). Second, regardless of the hypothesis about the origin of the face inversion effect is adopted, fish should not be configurally processed by carrion crows: if configural processing of faces is innate, only conspecifics should be relevant for crows, and if it is due to specialised expertise, fish should only be configurally processed by crows who have repeated exposure to fish and have a reason to differentiate between different species of fish. All pictures were presented both upright and inverted.

Behavioural protocol

Both crows had previously participated in other experiments using the same set-up and were thus habituated to the set-up and general procedure.

Matching-to-sample task

Several training steps were applied. First, the crows had to match colours (blue and red) and chromatic 'abstract' pictures taken from Veit and Nieder (2013) until they reached criterion (defined as accuracy >70%). In Step 2, birds had to match achromatic abstract patterns. In Step 3, birds had to match achromatic pictures of the same category (e.g., footballs). In Step 4, birds had to recognise two pictures of four different categories (mugs, tires, flowers, and keys).

Data collection

During data collection, six pairs of stimuli per class were used. Each correct test stimulus appeared once on the right and once on the left side of the screen, and each stimulus was twice the match stimulus and twice the non-match stimulus. Trial order was blocked, such that pictures of one category were blocked together. The order of blocks and trials within each block was randomised.

The crows were presented with a minimum of 192 correct trials during a session (4 different pairings per stimuli × 6 stimuli pairs × 2 orientations × 4 stimuli categories). Therefore, crows saw each picture at least 4 times during one session. During data collection, crows received between 384 and 576 trials each day (2–4 sessions).

Analysis

Data were extracted from CORTEX (National Institute of Mental Health) using MATLAB R2016a. For data analyses, a difference index was calculated for the percentage of correct responses on upright minus the percentage of correct responses on inverted trials (DI = Upright − Inverted). A face inversion effect would predict a larger impairment of the crows' performance when responding to face compared to non-face stimuli. Hence, the DI should be larger in face than non-face categories.

Data were analysed for each crow separately. First, the DI (as performance for upright stimuli minus the performance for inverted stimuli) when responding to crow faces was compared to the DI when responding to non-face controls (fish pictures), $DI_{crow\ face} > DI_{fish}$. Second, the DI when responding to human faces was compared to the DI when responding to non-face controls (house interior pictures), $DI_{human\ face} > DI_{house\ interior}$.

Whether overall performance differed from chance or not was analysed using a binomial test in RStudio Version 1.0.136 (R Core Team 2016). To assess the face inversion effect, the proportion of correct responses to all pictures of one category was calculated as one score for each category during each session. This was done for both upright and inverted stimuli separately. The comparisons between $DI_{crow\ face} > DI_{fish}$ as well as between $DI_{human\ face} > DI_{house\ interior}$ were analysed with paired Wilcoxon rank tests in RStudio. Comparisons based upon clear predictions were calculated using directional (one-sided) tests (Ruxton and Neuhäuser 2010).

All analyses were based upon clear predictions and as such all comparisons were calculated using directional (one-sided) tests (Ruxton and Neuhäuser 2010).

Investigating the face inversion effect in human participants (Experiment 2)

Participants

Twenty participants were recruited and tested at the Institute of Biology at the University of Tübingen, Germany, aged 21–35 ($M = 26.7$), of which 13 were females. The experiment was performed with the approval of the Ethical Committee of the Faculty of Science, University of Tübingen, performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Data were collected from January to February 2017.

Set-up and material

The same test stimuli as in Experiment 1 were used. The set-up was the same as in Experiment 1 except that the touch screen was moved to face the participants sitting in front of the box. The room was darkened. Piloting the original task on KFB and LW showed that humans were likely to perform at ceiling if the same timings as in the crow task were used. Thus, the presentation time of the sample was reduced to 500 ms and the delay between sample and test stimuli was increased to 500 ms. Furthermore, the available response time until a trial was aborted was reduced to 710 ms.

Procedure

Participants were instructed verbally. They were asked to complete 192 correct trials each. Similarly to the crows, humans received a retry for incorrect trials. The experiment took 20 min in total.

Analysis

Data were extracted from CORTEX (National Institute of Mental Health) using MATLAB R2016a and were analysed in RStudio Version 1.0.136 (R Core Team 2016). For data analyses a difference index was calculated for percentage of correct responses in upright minus inverted trials (DI = upright − inverted).

Due to non-normality, data were analysed using Wilcoxon signed rank tests. Because the analysis was based on clear predictions, directional tests were used. Cohen's *d*s were corrected for dependence according to Morris and DeShon (2002). First, the DI (as performance for upright stimuli minus the performance for inverted stimuli) when responding to crow faces was compared to the DI when responding to non-face controls (fish pictures), $DI_{crow\ face} > DI_{fish}$. Second, the DI when responding to human faces was compared to the DI when responding to non-face controls (house interior pictures), $DI_{human\ face} > DI_{house\ interior}$. Additionally, we

compared the performance on trials with crow faces with the performance on human faces, regardless of orientation.

Results

Assessing a possible face inversion effect in carrion crows

We first assessed the presence of a putative face inversion effect in crows (Experiment 1). Figure 3 gives the performance scores of the two crows for all categories in upright and inverted trials. Both crows performed the task better than chance (50%) for all stimulus categories (Binomial tests, all p's $< .001$). Crow Hugo scored on average $M = 86.9\%$ (SD = 9.1%) on trials with upright crow faces, and $M = 82.6\%$ (SD = 9.4%) on trials with inverted crow faces, $DI_{crow\ face} = 4.3\%$. He scored on average $M = 86.0\%$ (SD = 7.6%) on trials with upright non-face controls (fish), and $M = 80.6\%$ (SD = 7.6%) on trials with inverted non-face controls, $DI_{fish} = 5.4\%$. Hence, as can be seen in Fig. 4, Hugo did not show a face-specific inversion effect for crow faces, $U = 365.5$, $p_{one\text{-}sided} = .698$. With the human faces, he scored on average $M = 73.8\%$ (SD = 8.9%) on trials with upright human faces, and $M = 69.6\%$ (SD = 8.0%) on trials with inverted human faces, $DI_{human\ faces} = 4.2\%$. He scored on average $M = 88.1\%$ (SD = 7.8%) on trials with upright non-face controls (house interior), and $M = 79.1\%$ (SD = 7.8%), on trials with inverted non-face controls, $DI_{house\ interior} = 9.0\%$. Thus, Hugo also did not show a face-specific inversion effect for human faces, $U = 469$, $p_{one\text{-}sided} = .962$.

Crow Walt scored on average $M = 77.4\%$ (SD = 10.0%) on trials with upright crow faces, and $M = 74.1\%$ (SD = 10.5%) on trials with inverted crow faces, $DI_{crow\ face} = 3.3\%$. He scored on average $M = 76.0\%$ (SD = 9.9%) on trials with upright non-face controls (fish), and M = 75.3% (SD = 8.1%) on trials with inverted non-face controls, $DI_{fish} = 0.7\%$. This difference in DI did not reach significance, $U = 267$, $p_{one\text{-}sided} = .070$, see Fig. 3. With the human faces, he scored on average $M = 69.8\%$ (SD = 8.8%) on trials with upright human faces, and $M = 66.4\%$ (SD = 9.6%) on trials with inverted human faces, $DI_{human\ faces} = 3.3\%$. He scored on average $M = 78.8\%$ (SD = 9.5%) on trials with upright non-face controls (house interior), and $M = 75.8\%$ (SD = 9.5%), on trials with inverted non-face controls,

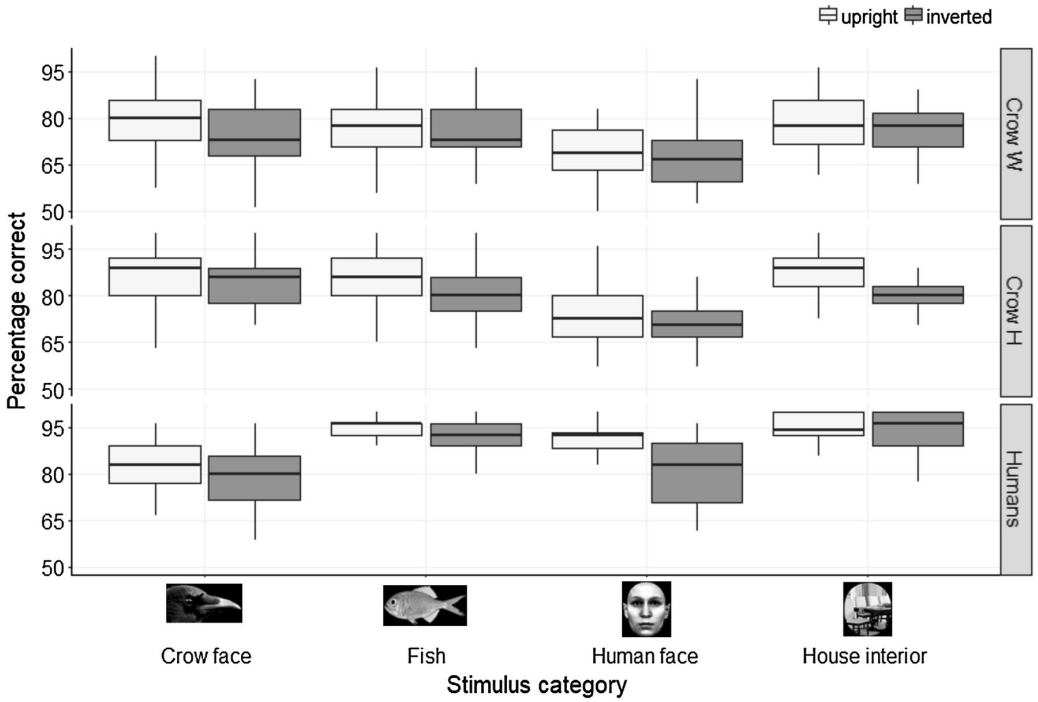

Fig. 3 *Box*-and-*whiskers* plot showing the performance for all stimulus categories when responding to upright stimuli (*light grey*) and inverted stimuli (*dark grey*) for crow Hugo ($n = 37$ sessions), crow Walt ($n = 39$ sessions) and the human participants ($n = 20$). The *boxes* signify the *upper* and *lower* quartiles and the *thick black horizontal lines* the median. The whiskers extend from the box to values no further than ±1.5 * IQR from the *box*

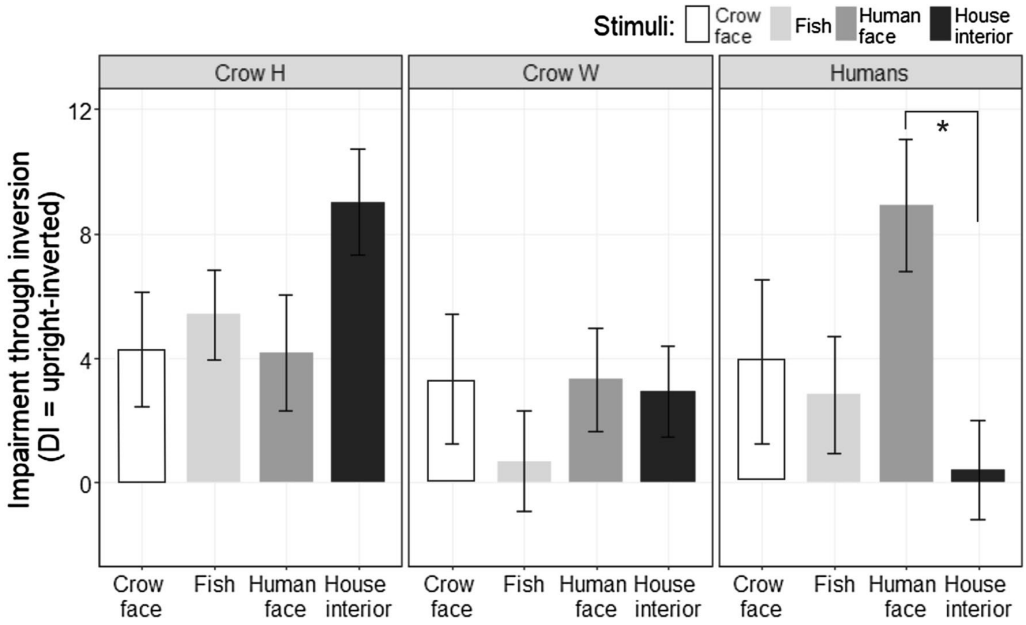

Fig. 4 Mean DI in performance ± SEM. Performance scores when responding to inverted pictures were subtracted from the performance scores when responding to upright pictures to determine the impairment due to inversion for the different stimulus categories, comparing crow Hugo, crow Walt and human participants. The *asterisk* indicates a significant difference (*$p = .001$, Wilcoxon-signed rank test)

$DI_{\text{house interior}} = 2.93\%$. Thus, Walt also did not show a face-specific inversion effect for human faces, $U = 226$, $p_{\text{one-sided}} = .339$.

Interestingly, as can be seen in Fig. 3, as well as in Table 1, both crows performed better when crow faces were presented compared to when human faces were presented, regardless of orientation (Hugo: $U = 2408.5$, $p_{\text{two-sided}} < .001$; Walt: $U = 2101$, $p_{\text{two-sided}} < .001$). Furthermore, both crows generally performed better when responding to upright than to inverted stimuli, regardless of category (Hugo: $U = 7785$, $p_{\text{two-sided}} < .001$; Walt: $U = 6667$, $p_{\text{two-sided}} = .004$).

Validation of the methodology with human participants

We validated the face inversion effect in humans using the same methodology (Experiment 2). The percentage of correct responses was on average $M = 82.5\%$ (SD = 11.1%) on trials with upright crow faces, and $M = 78.7\%$ (SD = 8.72%) on trials with inverted crow faces, $DI_{\text{crow face}} = 3.88\%$. On trials with upright non-face controls (fish), the percentage of correct responses was on average $M = 93.7\%$ (SD = 5.4%), and $M = 90.9\%$ (SD = 6.5%) on trials with inverted non-face controls, $DI_{\text{fish}} = 2.83\%$. This difference in DI did not reach significance, $U = 114.5$, $p = .222$ (Fig. 4). For the human faces, the average of percentage of correct responses was $M = 90.2\%$ (SD = 6.7%) on trials with upright stimuli, and $M = 81.3\%$ (SD = 10.9%) on trials with inverted stimuli, $DI_{\text{human faces}} = 8.91$. On trials with upright non-face controls (house interiors), the average of percentage of correct responses was $M = 94.5\%$ (SD = 4.5%), and $M = 94.1\%$

Table 1 Overview of performance (percentage of correct choice) for all stimulus categories for both birds and human participants, averaged across all sessions

	Mean (SD) performance in %		
	Crow Hugo	Crow Walt	Human participants
Fish	83.3 (8.0)	75.6 (9.0)	92.3 (6.1)
House interior	83.6 (8.8)	77.3 (9.0)	94.3 (5.8)
Crow face	84.7 (9.5)	75.8 (10.3)	80.6 (10.1)
Human face	71.7 (8.7)	68.1 (9.3)	85.8 (10.0)
Upright	83.7 (10.1)	75.5 (10.1)	90.3 (8.0)
Fish	86.0 (7.7)	76.0 (9.9)	93.7 (5.4)
House interior	88.1 (7.8)	78.7 (9.5)	94.5 (4.5)
Crow face	86.9 (9.1)	77.4 (10.0)	82.5 (8.7)
Human face	73.8 (8.9)	69.8 (8.8)	90.2 (6.7)
Inverted	78.0 (9.5)	72.9 (9.8)	86.2 (11.0)
Fish	80.6 (7.6)	75.3 (8.1)	90.9 (6.5)
House interior	79.1 (7.8)	75.8 (8.3)	94.1 (7.0)
Crow face	82.6 (9.4)	74.1 (10.5)	78.7 (11.1)
Human face	69.9 (7.9)	67.0 (9.5)	81.2 (10.9)
Overall	80.8 (10.2)	74.2 (10.0)	88.2 (9.8)

(SD = 7.0%), on trials with inverted non-face controls, $DI_{house\ interior} = 0.41\%$. The $DI_{human\ face}$ was significantly larger than $DI_{house\ interior}$, $U = 170.5$, $p = .001$. Hence, as can be seen in Fig. 4, humans showed a face inversion effect for human faces.

Discussion

In this study, we present results suggesting that carrion crows do not exhibit a face inversion effect. The face inversion effect refers to a pronounced impairment in the ability to recognise and remember faces compared to other stimuli once the pictures are turned upside-down (Yin 1969; Diamond and Carey 1986). As such, the face inversion effect has been suggested to reflect a special processing of faces.

The lack of a face inversion effect in carrion crows

In Experiment 1, we investigated whether carrion crows also show the face inversion effect or not, both with crow faces and with human faces. The crows performed better with upright than inverted stimuli in general, and their accuracy for inverted stimuli never reached the accuracy shown for upright stimuli. Some impairment following inversion is also found in humans (e.g., Diamond and Carey 1986; Experiment 2), and was previously reported for animals, too (e.g., Wright and Roberts 1996). However, note that this result could in part be explained by the fact that prior to data collection, when we selected the control stimuli, we exposed the crows to the upright examples of the respective category to assess which stimulus categories they were able to discriminate. One possible explanation for their difficulties to achieve similar performance for the inverted stimuli could be that they developed a strategy to respond to these pictures, which was rendered suboptimal once the pictures were inverted.

Furthermore, while not being the main focus of this study, it should be noted that crows were better at recognising crow faces compared to recognising human faces. However, neither of the crows tested showed a more pronounced impairment of their performance when presented with inverted faces—either human faces or crow profiles—compared to inverted control stimuli. Hence, the two crows tested did not show evidence of a face inversion effect.

There are three reasons why this lack of a face inversion effect in crows may be surprising. First, corvids can and need to identify specific individuals (e.g., Dally et al. 2006; Bugnyar 2011; Massen et al. 2015) and can do so from static pictures (e.g., Bird and Emery 2008; Braun 2013). Second, corvids can also recognise specific human faces (Marzluff et al. 2010; Clucas et al. 2013). And last, corvids can learn to discriminate pictures in general (Veit and Nieder 2013; Veit et al. 2014), and pictures of conspecifics in particular, as shown in Experiment 1. In the following, the lack of a face inversion effect in our crows is discussed in relation to the stimuli used and the cues crows (might) use to differentiate individuals.

Positive validation of the experimental procedures in human adults

In order to directly test whether the stimuli used could have been responsible for the lack of a face inversion effect in carrion crows, Experiment 2 validated the procedure and stimuli used in Experiment 1 by testing humans in the same set-up and with the same stimuli as the crows. Whether a face inversion effect is present in animals or not is still a matter of debate. It has been argued that the conflicting results reported regarding whether or not primates show a face inversion effect is due to differences in methods and stimuli used (Dahl et al. 2013). For example, some studies used natural pictures of full primate heads, sometimes with some scenery in the background (e.g., Parr et al. 1999; Phelps and Roberts 1994; Wright and Roberts 1996), while newer studies have used very controlled pictures, showing only a face without any surrounding that might allow viewers to determine head shape (Dahl et al. 2013). Thus, in Experiment 2, the paradigm and stimuli used in Experiment 1 were validated with a human sample. Here, humans showed a strong face inversion effect: their performance in recognising faces was impaired to a greater extent when pictures of human faces were inverted compared to pictures of non-face controls. This result is in line with a range of previous studies on the face inversion effect in humans (e.g., Yin 1969; Diamond and Carey 1986; Kanwisher et al. 1998; Freire et al. 2000; Turati et al. 2004). The result of Experiment 2 further suggests that, in principle, the stimuli used in our study are appropriate to induce a face inversion effect, as they do so in human participants. Consequently, the null result in Experiment 1 cannot be explained by a methodological problem and instead reflects a lack of a face inversion effect in the crows. The consistent results from the two crows suggest that this species does not show a face-specific inversion effect. However, given the small sample size in the current study, it remains a possibility that our results might not apply to crows in general.

Implications regarding the cues used by crows for individual recognition

Given the positive validation of the procedures used in Experiment 2, there are two possible reasons for a lack of face inversion effect in crows: first, crows might use and process cues other than face profiles to recognise and discriminate between conspecifics. It is not yet known whether crows

use facial cues to identify conspecifics. There are reports of certain bird species using facial cues to discriminate between conspecifics (e.g., Trillmich 1976; Brown and Dooling 1992; Nakamura et al. 2003), for example the diverse plumage of the face (Leopold and Rhodes 2010). It is, however, possible that crows in the wild use the whole body as a cue, rather than the face alone. Notably, research on conspecific discrimination in crows has so far mainly used whole bodies (Braun 2013). Thus, it would be of interest to see whether crows have a 'body-inversion' effect. Reed et al. (2003) found that humans display a body inversion effect in that their performance in recognising human bodies is impaired by inversion whereas recognition of houses is not.

Another cue that corvids might use for identity discrimination is ultraviolet differences in plumage. Ultraviolet light perception has been reported to be relevant for mate choice in a range of bird species (for a review see Rajchard 2009, but for opposing views see Stevens and Cuthill 2007). For example, Steller's jays' plumage UV reflection signals mate quality. Note, however, that extra-pair copulations play a relatively important role for Steller's jays, compared to other corvid species (Overeem et al. 2014). It is thus unclear whether the importance of UV perception in Steller's jays' sexual behaviour is indicative of visual features that might be relevant for monogamous and largely unassisted breeding carrion crows. Still, it is worth noting that the failure to find a face inversion effect might be due to the lack of UV light of the crow face stimuli used in the current study.

Second, the face inversion effect might simply not be an indicator for specialised processing in crows, maybe because crows do not process faces in a configural manner. In this case, a different approach might be necessary to evaluate whether faces are processed differently to stimuli of other categories. With electrophysiological experiments it has been demonstrated that rhesus macaques possess neural circuits specifically dedicated to processing faces (Allison et al. 2000; Gross 2008; Freiwald and Tsao 2010). Yet, studies investigating the face inversion effect in monkeys produced mixed results (e.g., Phelps and Roberts 1994; Wright and Roberts 1996; Parr 2011b). Thus, it has been argued that in monkeys, specialised face processing might not manifest itself in configural processing, which is susceptible to inversion (Leopold and Rhodes 2010). Consequently, it would be of interest whether electrophysiological experiments could uncover face-specific responses in the crow brain, too. Face-selective cells have been previously found in a range of primate species, from humans (Kanwisher and Yovel 2006) to macaques (Gross 2008; Freiwald and Tsao 2010) and marmoset (Hung et al. 2015), but also in a non-primate mammal, the sheep (Tate et al. 2006). Furthermore, faces seem to be special for another species of bird: newborn domestic chicks have been reported to show a predisposition to imprint on face-like stimuli (Johnson and Horn 1988; Rosa-Salva et al. 2010; Salva et al. 2011; Rosa Salva et al. 2012; Di Giorgio et al. 2016; Versace et al. 2017). It is thus possible that corvids, while not showing a face inversion effect, might have similar face-selective cells indicative of a specialised processing of faces.

Specialised processing of human faces by crows?

Previous research suggests that crows can use the face of a human to differentiate between individuals (Marzluff et al. 2010; Bogale et al. 2011) and can be trained to discriminate between male and female faces based on pictures (Bogale et al. 2011); therefore, we assessed the face inversion effect in crows for human faces as well. However, this prior research alone does not imply that human faces constitute a 'special' cue for crows. This notion is tentatively supported by the results presented here, because the birds did not show an inversion effect when presented with human faces, suggesting that crows might use local features to differentiate them. Such feature recognition would not be impaired by inversion. There are of course a range of different features they could have used, such as for example the shape or size of the eyes. Future research is needed to assess whether they indeed used local features to solve the matching-to-sample task, and if so, which ones.

Conclusion

In summary, our results suggest that crows do not exhibit a face inversion effect. We further show that crows can learn to discriminate between human as well as crow faces, and make fewer errors when responding to crow faces. Based on the rationale from human and other primate studies, these findings may be taken to mean that crows are no 'experts' for faces and thus do not process faces in a different way to other stimuli. Further research is needed to determine which cues crows use to differentiate between different conspecifics as well as humans, and whether there are other ways to assess a possible specialised processing of faces in crows.

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Study 4: Neurons in the endbrain of numerically naive crows spontaneously encode visual numerosity

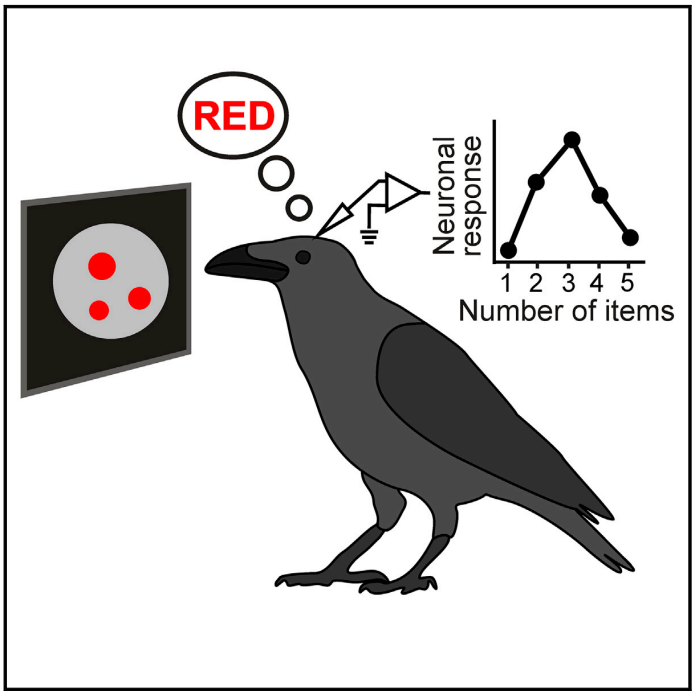
Wagener, L., Loconsole, M., Ditz, H. M., Nieder, A. (2018) Neurons in the endbrain of numerically naive crows spontaneously encode visual numerosity. *Current Biology*, 28(7), 1090-1094.

Report

Current Biology

Neurons in the Endbrain of Numerically Naive Crows Spontaneously Encode Visual Numerosity

Graphical Abstract



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In Brief

In crows never trained to assess numerical quantity, Wagener et al. found neurons in the nidopallium caudolaterale that were spontaneously selective for the number of items. Hard-wired neuronal connections for numerical information are not only present in the cerebral cortex but also in the avian endbrain that evolved by convergent evolution.

Highlights

- NCL neurons respond to numerosity in numerically naive crows
- Numerosity-selective neurons were tuned to the number of items
- Numerosity-selective neurons are spontaneously present in the avian endbrain
- Convergent evolution of neuronal number code in different vertebrate endbrains

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Neurons in the Endbrain of Numerically Naive Crows Spontaneously Encode Visual Numerosity

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SUMMARY

Endowed with an elaborate cerebral cortex, humans and other primates can assess the number of items in a set, or numerosity, from birth on [1] and without being trained [2]. Whether spontaneous numerosity extraction is a unique feat of the mammalian cerebral cortex [3–7] or rather an adaptive property that can be found in differently designed and independently evolved neural substrates, such as the avian endbrain [8], is unknown. To address this question, we recorded single-cell activity from the nidopallium caudolaterale (NCL), a high-level avian association brain area [9–11], of numerically naive crows. We found that a proportion of NCL neurons were spontaneously responsive to numerosity and tuned to the number of items, even though the crows were never trained to assess numerical quantity. Our data show that numerosity-selective neuronal responses are spontaneously present in the distinct endbrains of diverge vertebrate taxa. This seemingly hard-wired property of the avian endbrain to extract numerical quantity explains how birds in the wild, or right after hatching, can exploit numerical cues when making foraging or social decisions. It suggests that endbrain circuitries that evolved based on convergent evolution, such as the avian endbrain, give rise to the same numerosity code.

RESULTS

Whether humans and animals are endowed with an innate faculty to perceive the number of items in a set (that is, numerosity) is intensely discussed. The idea of a "number sense" [12, 13] argues that numerosity is assessed intuitively as a spontaneous category by hard-wired brain processes, without the need to be learned. Support for the direct and spontaneous assessment of numerosity resulted from psychophysical experiments in humans showing that approximate visual number assessments are subject to adaptation [3, 4]. In addition, recent imaging evidence suggests that the direct and automatic extraction of numerosity also occurs in the human brain [5, 6]. The most direct support for the notion of a "number sense" comes from recordings in monkeys that had not been trained to judge number; these recordings showed that single neurons in both the parietal and prefrontal cortices spontaneously responded to numerosity and were tuned to preferred numerosities [7].

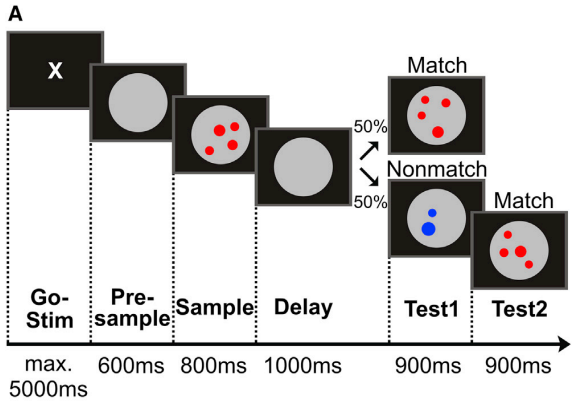
However, all of these data have been collected in primate species that possess an elaborate six-layered cerebral cortex as highest integration center in the brain. Whether spontaneous numerosity extraction is a special feature of the cerebral cortex or rather an adaptive property that can be found in differently designed and independently evolved endbrains is unknown.

We therefore investigated the question of spontaneous numerosity selectivity in a bird species: the carrion crow. Instead of a cerebral cortex, birds possess nuclear telencephalic areas [8] as highest integration centers that evolved independently since the last common reptilian-like ancestor of birds and mammals lived 320 million years ago [14]. We recently showed that neurons in the endbrain region nidopallium caudolaterale (NCL), a brain area considered to be the avian analog of the primate prefrontal cortex [9–11], respond selectively to the number of visual items in numerically trained crows [15, 16]. In the current study, we explored spontaneous neuronal selectivity to numerosity in crows that had never been trained to discriminate the number of items in a set.

Crows Performed the Color Discrimination Task and Were Ignorant of Numerosity

Two crows (*Corvus corone*) were trained to discriminate color in variable dot displays in a delayed match-to-sample (DMS) task. This ensured that the crows paid attention to the stimulus displays during recording (Figure 1A). The crows saw two colored-dot displays (first sample, then test) separated by a 1 s delay. They were trained to respond by moving their head whenever the (1–5) dots in the sample and test displays were of the same color. Five colors (red, blue, green, yellow, purple) were used (Figure 1B). Importantly, the crows were only trained to discriminate color, not numerosity. All five colors and numerosities were displayed as "standard stimuli," with variable dot sizes and positions, and "control stimuli" equating the total area and the average density of all dots across numerosities. All parameters (color, numerosity, stimulus protocol, match versus non-match trials, etc.) were balanced and pseudo-randomly presented in each session.

Both crows performed the color-discrimination task proficiently well above the 50% chance level (crow T: 99% ± 0.2% SEM, n = 50 sessions; crow V: 95% ± 0.3% SEM, n = 43 sessions; Figure 2A) for all sample colors (all binomial tests, $p < 0.001$). To ensure that the crows had indeed discriminated color and not numerosity, we inserted a small fraction of



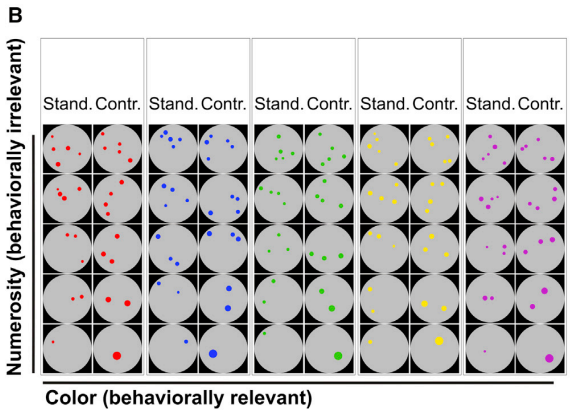


Figure 1. Task Protocol and Example Stimuli

(A) The crows performed a delayed match-to-sample task in which they discriminated the color of dot arrays. A trial was initiated by moving the head into a light barrier in front of the screen and keeping it in this position. After a short pre-sample phase, a sample stimulus (colored-dot array) was presented for 800 ms, followed by a delay of 1,000 ms. In the subsequent test phase, a match stimulus (same color as the sample) was shown as test 1 in 50% of the trials, in the other half a non-match stimulus (different color as the sample) was presented first and followed by a match stimulus. The crow was rewarded for responding by moving its head out of the light barrier whenever the color of a test stimulus matched the color of the sample.

(B) Example stimulus displays. Each of the five colors was presented in five different numerosities and two different stimulus sets (standard and control).

generalization trials during the ongoing color-discrimination task. In generalization trials, the dots of both sample and test stimuli were all black. If the crows were ignorant of numerosity and relied on color, they would perform at chance level for the all black dot arrays. Indeed, both crows performed at chance level in black-dot trials (crow T: 52%, n = 283 trials; crow V: 52%, n = 270 trials; both binomial tests, $p \geq 0.5$; Figure 2B).

Neurons Spontaneously Tuned to Numerosity

We recorded the activity of 403 single neurons (crow T: 289; crow V: 114) in the NCL (Figure 3A) while the crows performed the color-discrimination task with colored-dot stimuli. We found

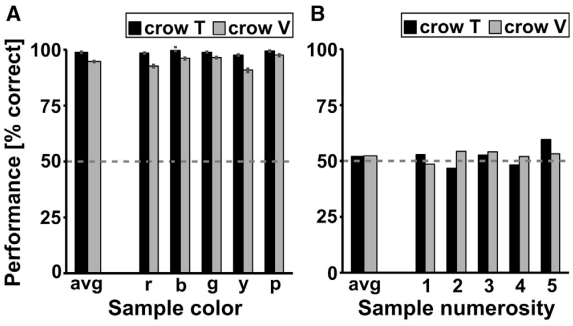


Figure 2. Behavioral Performance of Both Crows

(A) Performance in the color discrimination task during recording sessions (crow T: n = 50; crow V: n = 43). Chance level is 50%. Error bars indicate SEM across the sessions.

(B) Performance in the numerosity-discrimination task in the generalization test sessions (crow T: n = 283 trials; crow V: n = 270 trials). Chance level is 50%.

cells that responded differently to specific numbers of dots (i.e., numerosities) during the sample presentation. Figure 3 shows the activity of three exemplary neurons. The example neuron in Figure 3B showed the highest activity to numerosity 1, whereas the other neurons responded strongest to numerosity 2 (Figure 3C) and 5 (Figure 3D).

A three-factor ANOVA (numerosity × color × protocol) was used to statistically test the neurons' selectivity to the different stimulus parameters. Neurons that showed a significant main effect for numerosity ($p < 0.01$), but no significant main effect for protocol or any interaction, were identified as numerosity-selective neurons and considered for further analyses. The behaviorally irrelevant parameter "numerosity" significantly modulated the activity of 12% (48/403) of the NCL neurons. Of those 48 numerosity-selective cells, 19 neurons (39.6%) showed an additional main effect for color. All neurons depicted in Figure 3 were numerosity selective according to this criterion. Table S1 shows the proportions of neurons that were significant to each of the main factors and interactions. These proportions of significant neurons are well beyond the chance level of about 1% of selective cells that we got when the spike rates of individual neurons were shuffled and analyzed in the same way (Table S2).

These neurons were tuned to the number of dots; they showed the highest discharge rates to a specific numerosity, its preferred numerosity, and a progressive decay of activity for neighboring numerosities (see tuning curve insets in Figures 3B–3D). Most of the selective neurons preferred numerosity 1 and 5; fewer neurons were tuned to the other intermediate numerosities (Figure 4A). Note that an increased frequency count for preferred numerosity 5 is even expected as the tested numerosity range was truncated to numerosity 5, and few neurons assigned to this class may, in fact, have been tuned to numerosities larger than 5.

To create average neural filter functions, activity rates were normalized by setting the maximum activity to the most preferred numerosity as 100% and the activity to the least preferred numerosity as 0%. Tuning functions to each of the sample numerosities were constructed by averaging the normalized spike rates

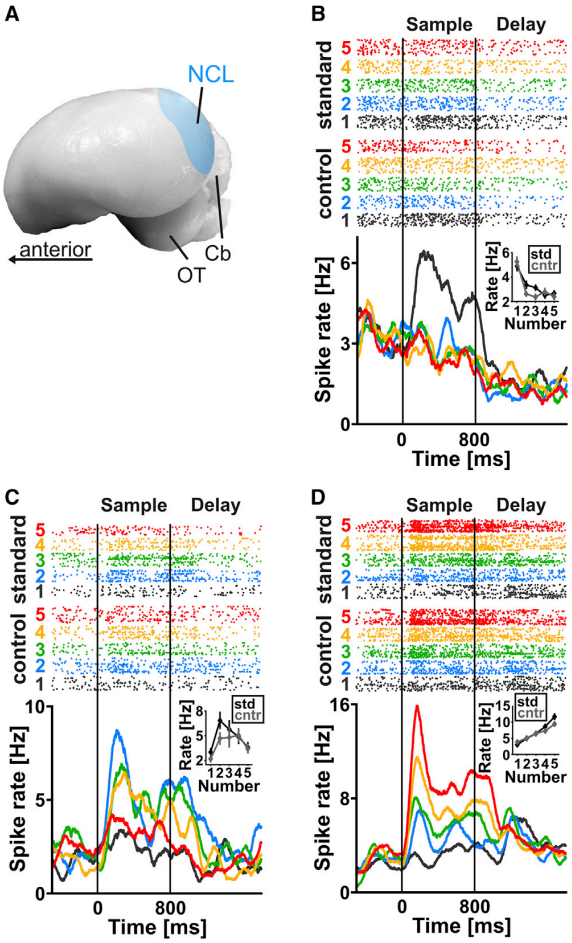


Figure 3. Brain Area and Neuronal Responses

(A) Lateral view of a crow brain with the nidopallium caudolaterale (NCL) located inside the telencephalon color coded. Cb, cerebellum; OT, optic tectum.

(B–D) Neuronal responses of exemplary neurons to the number of presented dots in the sample stimulus. The neurons were selective to numerosity 1 (A), 2 (B), and 5 (C). Top: Dot-raster histograms with each line indicating one trial and each dot representing an action potential. Activity is separated for standard and control conditions. Bottom: Corresponding spike-density functions, representing the time course of the average response to each numerosity (smoothed by a 150 ms Gauss kernel). Colors of dot-raster histograms and spike-density functions correspond to the numerosity of the sample stimulus. Vertical line at 0 ms indicates onset of the sample that was shown for 800 ms. Tuning function insets indicate the average firing rate to numerosity in the standard (std) and control (cntr) condition. Error bars represent SEM. See also Tables S1 and S2.

of all neurons that had the same preferred numerosity. This resulted in overlapping numerosity tuning curves (Figure 4B). Across the population, NCL neurons covered the entire tested range of numerosities 1–5. Finally, we plotted the average normalized activity across the population of numerosity-selective neurons as a function of the numerical distance from the preferred numerosity (Figure 4C). On average, neuronal activity dropped as a function of the numerical distance from the preferred numerosity, a neuronal correlate of the "numerical distance effect" that has been reported for numerosity-selective NCL neurons in trained crows [15, 16].

DISCUSSION

In the current study, we tested the core idea of the "number sense" and explored, for the first time in a non-primate species, whether numerosity-selective neurons spontaneously exist in the brain of crows. To that aim, we recorded single-cell activity from the NCL, a high-level avian association brain area [17–19], of numerically naive crows. We show that a proportion of NCL neurons is selectively tuned to the number of items in a set. This demonstrates that numerosity-selective neurons are not the result of behavioral training but spontaneously exist in crows that have never been trained to discriminate numerosity.

Without numerosity training, we found that 12% of NCL neurons responded selectively to the number of presented dots. This proportion was significantly smaller compared to the 20% of numerosity-selective neurons from the same NCL region in crows trained to perform a numerosity-discrimination task [15] (chi-square tests, $p < 0.01$). However, the selectivity of the numerosity-selective responses was comparable for data from naive and trained crows. We compared the widths of the numerosity-tuning curves as measured by sigma of Gauss-fits to the (logarithmically scaled) tuning functions [20] and found no difference between numerically naive and trained crows (Mann-Whitney-U test, $p = 0.86$). Based on these comparisons, we conclude that numerosity training may increase the proportion of numerosity-selective cells in NCL but not their coding properties.

The only other animal species for which single-unit data about numerosity coding is available are macaque monkeys. In these primates, the ventral intraparietal area (VIP) and prefrontal cortex (PFC) have been identified as key areas for number representations [21, 22]. Interestingly, the proportion of selective neurons (12%) in the NCL of numerically naive crows is almost identical to the 13% and 14% of numerosity-selective neurons in the VIP and PFC, respectively, of numerically naive monkeys [7]. This suggests the NCL as a neuronal substrate for representing numerical information, much in the way as the VIP and PFC constitute the core number system in primates.

Our study also speaks to the question of the neuronal code for numerical quantity in the animal kingdom. Two competing hypotheses have been proposed. Numbers could either be encoded by a "summation code" as witnessed by monotonic discharges as a function of quantity [23], or by a "labeled-line code" as evidenced by neurons tuned to preferred numerosities [21]. In agreement with influential computational models of number processing [24, 25], the numerosity-selective neurons we found in the NCL of numerically naive crows were tuned to their individual preferred numerical value. The same code has been found in numerically trained crows [15, 16] and multiple times in single-cell recordings in monkeys, both trained [26–31] and numerially naive [7]. It therefore seems that the neuronal code for number information is a labeled-line code. This code seems to have

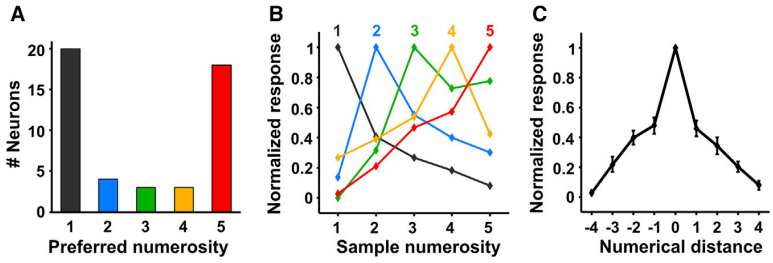


Figure 4. Average Tuning Properties

(A) Frequency distribution of the neurons' preferred numerosities.

(B) Average tuning functions for neurons preferring the same numerosity.

(C) Average normalized activity of all numerosity-selective neurons as a function of numerical distance from the preferred numerosity. Error bars indicate SEM.

evolved independently in phylogeny in birds and mammals, two distantly related vertebrate taxa [32]. The labeled-line code may be computationally superior when compared to alternative neuronal representations such as summation coding.

The ability to spontaneously assess the number of items in an approximate way is widespread across the animal kingdom, indicating that it is of adaptive value. Tests in which animals can choose between sets of food objects show that different species spontaneously "go for more" and pick the sets containing more food items [33–37]. Similarly, animals in the wild spontaneously exploit quantitative information in social interactions [2, 38, 39]. For these animals to successfully discriminate set size, numerosity-selective neurons must spontaneously be implemented in their brains. Without such neurons, they could not solve such numerical tasks in the first place.

The current data in crows together with a report about numerosity-selective neurons in the parietal and prefrontal cortex of monkeys [7] argue that the neuronal mechanisms for approximate number discrimination are readily available without number training in differently designed endbrains. This begs the question whether animals might be born with hard-wired neuronal networks that can represent numerical information. Alternatively, numerosity selectivity could emerge implicitly as a function of increased visual experience with different numbers of objects throughout development. To address this question directly, recordings in juvenile crows at the moment of eye opening would be necessary. However, even without such data, behavioral investigations suggest that numerical competence is present from early on in birds.

The young domestic chick is an extremely precocial species and has been tested for numerical competence right after hatching from the egg and thus with a minimum of visual experience. Exploiting filial imprinting few hours after hatching, chicks have been shown to discriminate numerosity and even perform rudimentary arithmetic [40, 41]. Moreover, newborn human infants at the age of 50 hr also discriminate abstract numerosity, even across sensory modality and sequential and simultaneous presentation formats [1].

All of these data together argue that numerosity selectivity may indeed be inborn, not only in primates but also in other vertebrates. This suggests that hard-wired (but, of course, modifiable) neuronal connections extracting numerical information are not a special property of the cerebral cortex but are also implemented in the anatomically distinct endbrain circuitries of birds that evolved based on convergent evolution. How these distinct endbrain designs give rise to the same type of numerosity code needs to be addressed in the future.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Behavioral analysis
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- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables and can be found with this article online at https://doi.org/10.1016/j.cub.2018.02.023.

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AUTHOR CONTRIBUTIONS

L.W., H.M.D., and A.N. designed the experiment. L.W. and M.L. conducted the experiments. L.W. analyzed the data. L.W. and A.N. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing financial interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Corvus corone	University of Tübingen, Institute of Neurobiology	Crow T, crow V
Software and Algorithms		
NIMH Cortex	National Institute of Mental Health	c598; https://www.nimh.nih.gov/labs-at-nimh/research-areas/clinics-and-labs/ln/shn/software-projects.shtml
MAP Data Acquisition System	Plexon	https://plexon.com/
R2013b	MathWorks	https://www.mathworks.com
Other		
Dental Cement	Heraeus	Paladur, ISO 20795, CE 0197
Microdrives	Animal Physiology Unit	Custom fabrication
Electrodes	Alpha Omega LTD	Cat.#: 366-130620-00; www.alphaomega-eng.com

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Andreas Nieder (andreas.nieder@uni-tuebingen.de).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Carrion crows

Two hand-raised male carrion crows (*Corvus corone corone*, 6 and 2 years old) were used in this experiment. The birds were housed in social groups in indoor aviaries. They were on a controlled feeding protocol during the training and recording period. Body weight was measured daily. The daily amount of food was given as reward during, or if necessary after, the sessions. Water was *ad libitum* available in the aviaries and during the experiments. All procedures were carried out according to the guidelines for animal experimentation and approved by the responsible national authorities, the Regierungspräsidium Tübingen, Germany.

METHOD DETAILS

Apparatus

The birds were placed on a wooden perch in front of a touchscreen monitor (ART development PS-150, 15″, 60 Hz refresh rate) in a darkened operant conditioning chamber. The CORTEX system (National Institute of Mental Health, MD, USA) was used to control the stimulus display on the screen and to store the behavioral data. An infrared light barrier ensured, controlled by a reflector foil attached to the bird's head, a stable head position in front of the screen throughout the trial and was used as the response instrument by the bird. A custom-built automated feeder below the screen delivered either mealworms (*Tenebrio molitor* larvae) or bird seed pellets as reward upon correctly completed trials. Additionally, the birds received specific auditory feedback sounds for correct and error trials.

Stimuli

The visual stimuli were generated using a custom-written MATLAB software. They consisted of a colored dot array presented on a gray background circle. Each combination of five colors (red, blue, green, yellow, purple) and five numerosities (1, 2, 3, 4, 5) was used (Figure 1B). For the generalization test, black dot arrays were used. To prevent the crows from memorizing the visual patterns of the dot arrays, a new stimulus set with four different images for each color-numerosity combination was generated for each session.

For the standard stimuli, the diameter of each dot varied randomly within a given range. In addition, control stimuli controlling for total dot area (the total area of all dots in a display was equal for all stimuli within a trial) and dot density (mean distances between centers of the dots in a display was equal for all stimuli within a trial) were used in each session. Trials containing standard or control stimuli were pseudo-randomly shuffled and equally likely to occur.

Behavioral protocol

The crows performed a delayed match-to-sample (DMS) task in which they discriminated the color of dot arrays (Figure 1A). A trial was initiated by positioning the head facing the monitor whenever a go-stimulus (small white cross) was shown, thus closing an infrared light barrier, and maintaining this position throughout the trial. To indicate that the light barrier had been entered, the bird

heard a click sound and the go-stimulus turned into a small white circle for 60 ms. Whenever a crow made premature head movements and thereby left the light barrier during an ongoing trial, this trial was terminated and discarded. In the 600 ms pre-sample phase, a plain gray background circle was shown in the center of the screen. Then the sample dot array was presented within the background circle for 800 ms. The color and numerosity of the dot array were pseudo-randomly selected. During the subsequent 1000 ms delay, only the plain background circle remained on the screen. In the following test phase, another dot array, the test1 stimulus, was presented for 900 ms. It was a 'match' in 50% of the cases, i.e., the dot array had the same color and numerosity as the sample, however it was never exactly the same image. The crow had to respond by moving its head out of the light barrier to receive a reward. In the other half of the cases, the test1 stimulus was a 'nonmatch' showing a dot array of another color and numerosity as the sample. Here, the crow had to refrain from responding and wait until the test2 stimulus, which was always a 'match', appeared. Responses to the 'nonmatch' stimulus and no response to either of the two test stimuli were considered as error trials and therefore not rewarded.

Generalization test

To confirm that the crows discriminated the stimuli based on color and not on the irrelevant parameter numerosity, we tested them with pure numerosity stimuli (black dot arrays, numerosity 1 to 5). These trials contained no color information (sample and test stimuli black) and were randomly inserted during the ongoing color discrimination task. The ratio of generalization trials was between 12.3% and 17.1% of the total number of trials. Reward was given for correctly solved numerosity trials (i.e., responding to the test stimulus which showed the same numerosity as the sample), however the birds were not forced to solve these trials correctly. Three generalization test sessions without neural recording were done for each bird: before, during and after the recording period.

Surgery and neuronal recordings

The surgery was performed while the animal was under general anesthesia with a mixture of ketamine (50 mg/kg) and Rompun (5 mg/kg xylazine). The head was placed in a stereotactic holder. To locate the target region, stereotaxic coordinates (center of craniotomy: AP 5 mm, ML 13 mm) were used. Neurons were sampled a few millimeters around these coordinates.Two custom-built microdrives with four glass-coated tungsten microelectrodes (2 MΩ impedance, Alpha Omega LTD, Israel) each and a connector for the head stage were chronically implanted. The eight electrodes were located in the NCL of the left hemisphere of crow T and the right hemisphere of crow V. No clustering of numerosity selectivity was detected across electrodes or recordings depths. A small head post for the reflector of the light barrier was already implanted in the course of previous experiments. After the surgery, the birds were provided with postoperative analgesics (Morphasol, 1 mg/kg butorphanol).

Each recording session started with adjusting the electrodes until a proper neuronal signal was detected on at least one channel. The neurons were never pre-selected for any involvement in the task. Single-cell separation was done offline (Plexon Offline Sorter, version 2.6.2).

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral analysis

Data analysis was performed using MATLAB (MathWorks, R2013b). The behavioral performance, which quantifies the ratio of correct answers, was calculated as the number of correct trials divided by the total number of trials. For the color discrimination task, the performance was calculated for each sample color in each session, statistically verified using a binomial test, and averaged across all sessions. To exclude numerosity discrimination, the performance for each numerosity in trials with black dot arrays during the generalization sessions (trials of the three sessions added together) was calculated and tested using a binomial test.

Neuronal analysis

The analyzed neuronal data included all cells that were recorded for at least 20 correct trials of each sample color and numerosity and had an average firing rate higher than 1 Hz during the entire trial. Neuronal responses to the sample stimulus were analyzed in an 800 ms window shifted by 100 ms from stimulus onset to account for response latency.

To identify numerosity-selectivity, defined as a difference in firing rate as a function of the number of presented dots, a three-factor ANOVA with main factors sample numerosity (1, 2, 3, 4, 5), sample color (red, blue, green, yellow, purple) and protocol (standard or control) was performed. A neuron was classified as numerosity-selective if it showed either a significant main effect for numerosity ($p < 0.01$) or for numerosity and color, but no significant effect for protocol and interactions. The preferred numerosity was defined as the numerosity which elicited the highest firing rate. We compared the proportion of selective neurons found in the real data with shuffled firing rate data as a measure of chance selectivity. Data were shuffled a thousand times per neuron and each time tested with the three-factor ANOVA.

To derive average tuning functions of the numerosity-selective neurons, the individual tuning functions were normalized by setting the highest firing rate to the preferred numerosity as 100% and the lowest firing rate as 0%. These were then averaged across all neurons which preferred the same numerosity and as a function of the numerical distance from the preferred numerosity, respectively.

To evaluate potential changes in the selectivity of numerosity tuning in naive versus numerically trained crows, we compared the width of the tuning functions in naive crows (this dataset) with a previously recorded dataset in numerically trained crows [15]. To that

aim, Gauss-functions were fit to the neuronal tuning functions of each numerosity-selective neuron. The Gaussian was chosen because it represents the standard symmetric distribution and, thus, provided a means to compare the tuning functions. Data were plotted on a logarithmic scale because this provides symmetric tuning functions [15]. The derived width (sigma) of the Gauss fits was then compared between data in naive and trained crows.

DATA AND SOFTWARE AVAILABILITY

Analysis-specific code and data are available by request to the Lead Contact.

Current Biology, Volume *28*

Supplemental Information

Neurons in the Endbrain of Numerically Naive Crows Spontaneously Encode Visual Numerosity

Lysann Wagener, Maria Loconsole, Helen M. Ditz, and Andreas Nieder

Table S1. Related to Figure 3; Neuronal selectivity to different task factors

Number & percentage of cells selective for	
Numerosity	70/403 (17.4%)
Color	85/403 (21.1%)
Protocol	17/403 (4.2%)
Interaction Numerosity x Color	12/403 (3.0%)
Interaction Numerosity x Protocol	26/403 (6.5%)
Interaction Color x Protocol	5/403 (1.2%)

Table S2. Related to Figure 3 and Table S1; Chance selectivity based on shuffled neuronal data (1000 permutations per neuron)

Percentage of cells selective for	
Numerosity	1.00%
Color	0.99%
Protocol	1.01%
Interaction Numerosity x Color	1.02%
Interaction Numerosity x Protocol	0.99%
Interaction Color x Protocol	0.99%

Study 5: Categorical representation of abstract spatial magnitudes in the executive telencephalon of crows

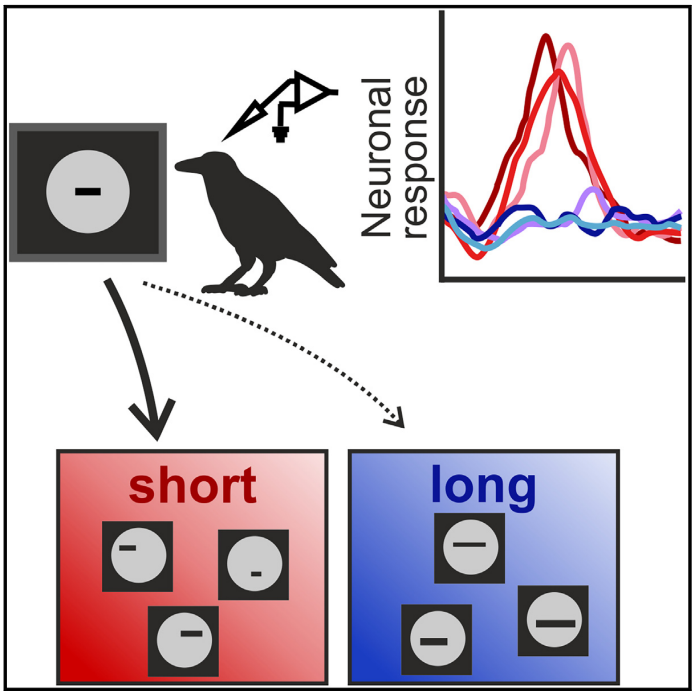
Wagener, L., Nieder, A. (2023) Categorical representation of abstract spatial magnitudes in the executive telencephalon of crows. *Current Biology* 33(11), 2151-2162.

Article

Current Biology

Categorical representation of abstract spatial magnitudes in the executive telencephalon of crows

Graphical abstract



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In brief

Wagener and Nieder show that neurons in the NCL of crows trained to group lines into "short" and "long" categories reflected these magnitude categories in a behaviorally relevant way. Neuronal category representations changed flexibly after retraining a crow with identical stimuli to new categories "short", "medium", and "long".

Highlights

- Crows classified lines in a match-to-sample task into "short" and "long" categories
- NCL neurons encoded category information and category boundaries
- NCL activity changed with retraining to reflect new length categories
- Malleable categorization is mediated by the flexible networks of the crow NCL

Wagener & Nieder, 2023, Current Biology *33*, 2151–2162
June 5, 2023
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Article

Categorical representation of abstract spatial magnitudes in the executive telencephalon of crows

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SUMMARY

The ability to group abstract continuous magnitudes into meaningful categories is cognitively demanding but key to intelligent behavior. To explore its neuronal mechanisms, we trained carrion crows to categorize lines of variable lengths into arbitrary "short" and "long" categories. Single-neuron activity in the nidopallium caudolaterale (NCL) of behaving crows reflected the learned length categories of visual stimuli. The length categories could be reliably decoded from neuronal population activity to predict the crows' conceptual decisions. NCL activity changed with learning when a crow was retrained with the same stimuli assigned to more categories with new boundaries ("short", "medium," and "long"). Categorical neuronal representations emerged dynamically so that sensory length information at the beginning of the trial was transformed into behaviorally relevant categorical representations shortly before the crows' decision making. Our data show malleable categorization capabilities for abstract spatial magnitudes mediated by the flexible networks of the crow NCL.

INTRODUCTION

Perceptual categorization enables animals to group stimuli into behaviorally meaningful classes that can easily be generalized to new circumstances.[1] Variable stimuli are distinguished as belonging to the same category (within category) or to different categories (across category). Even if the sensory features of to be categorized stimuli change continuously, the classification judgment from one category to another is sudden, thus resulting in an abrupt category boundary.[2]

In some animals and domains, the categorical perception of stimuli can be largely innate. For example, female túngara frogs respond categorically to complex male mating calls,[3] crickets divide sound frequency categorically into attractive and repulsive sounds,[4] and lactating female house mice perceive the ultrasonic calls of their pups categorically.[5] In many other circumstances, however, perceptual categories need to be learned by trial-and-error based on behavioral feedback.[6] For instance, young vervet monkeys need to learn to identify the predator category alarm calls,[7] and songbirds learned to recognize new alarms by association with known alarms.[8] Evidently, the capability to categorize stimuli offers survival and reproduction benefits and therefore is widespread across the animal kingdom.[9]

Experience-dependent categorization is frequent in cognitively flexible vertebrates. It can be found in mammals[10,11] and birds such as pigeons[12–19] and crows.[20–24] Similar to the hierarchical processing pathway in the primate brain,[25] behaviorally relevant stimulus features supporting categorical neuronal responses seem to be extracted gradually along the two major visual forebrain pathways of birds[26]: the thalamofugal pathway (homolog to the mammalian geniculocortical pathway) and the tectofugal pathway (thought to be analogous to the mammalian extrastriate cortex[27]). In the avian telencephalon, rudimentary category representations emerge first via the thalamofugal pathway in the thalamorecipient structures of the visual Wulst and via the tectofugal pathway in the entopallium and the overlaying intercalated nidopallium (NI) and mesopallium ventrolaterale (MVL) layers.[28,29] From these layers, highly integrated visual information still lacking sufficient feature invariance is forwarded to the dominant associative cognitive control center of the avian brain, the nidopallium caudolaterale (NCL). Based on a variety of anatomical and functional criteria, the NCL is thought to be an avian equivalent of the primate prefrontal cortex (PFC),[30–36] a mammalian brain area of great importance in categorization.[11,37–40]

Neuronal responses that establish behaviorally relevant conditional stimulus-response contingencies have been reported several times in the avian NCL.[18,41–44] Clear categorical neuronal responses were observed in the realm of numerical quantity. NCL neurons are tuned to the number of items in visual displays, both in numerically trained crows[45–48] but also in numerically naive crows[49] and untrained 10-day-old domestic chicks.[50] The latter findings suggest that categorical responses to number emerge largely spontaneously based on mechanisms inherent to the visual system.[51,52] How learned magnitude categories emerge in the avian brain and the neuronal mechanisms underlying them is currently unknown.

Here, we explored crows' behavioral and neuronal representation of learned magnitude categories. We tested three assumptions: first, we hypothesized that neurons in the corvid NCL represented learned and abstract spatial categories in a stimulus feature invariant and behaviorally relevant manner. Therefore, we trained crows in a delayed match-to-category task to group

the lengths of parameter-controlled lines into the categories "short" vs. "long" by relying on learned and arbitrary rules while recording from neurons of the NCL during performance. Second, we assumed that NCL neurons can flexibly adapt to new category boundaries if categorization rules change. Therefore, we retrained one crow with the line lengths reassigned to three new categories short, medium, and long. Third, we predicted that crow NCL neurons, despite a distinct neuroanatomy, exhibit a similar code for categories as PFC neurons in monkeys. Similarities of crow NCL data with monkey PFC findings would lend support to the notion of a superior physiological solution to the same categorization challenge in convergently evolved telencephalic executive brain regions.

RESULTS

Two crows were trained in a delayed match-to-category task to categorize line stimuli according to their length into two groups (short and long categories). Six different line lengths were used that were assigned to the two length categories short (S1, S2, and S3) vs. long (L1, L2, and L3) (Figure 1B). To ensure that the crows categorized length rather than the area or thickness of the lines, we used two stimulus protocols ("standard," where line thickness varied pseudo-randomly across line lengths, and "control," where the area of each line was constant) in each session.

Behavior

Both crows were able to memorize and match the sample line length to the category-matching length in the test phase. The crows performed proficiently above the 50% chance level (crow 1: 87.2% ± 0.5% SEM, n = 52 sessions; crow 2: 87.7% ± 0.7% SEM, n = 55 sessions) in each session (all binomial tests, $p < 0.001$). The behavioral performance was a step function with similar responses for stimuli of the same category and a sharp change across the category boundary (Figures 1C and 1D). Both crows reliably categorized each of the six individual sample stimuli to the appropriate length category, irrespective of whether standard or control protocols were shown (Figures 1E and 1F).

As expected for parameterized length magnitude, both crows categorized the lengths most distant from the category boundary (S1 and L3, respectively) most proficient and the lengths near the category transition (S3 and L1, respectively) least proficient, resulting in performance differences of between 2.6% and 19.7% between the most distant and the closest line length to the category boundary (S1 vs. S3 and L3 vs. L1, respectively) (Kruskal-Wallis tests, $p < 0.001$; except for short stimuli in match conditions for crow 1 ($p = 0.16$), Figure 1C, left). However, this within-category performance drop was mild compared with the substantial across-category (short vs. long) difference of, on average, 76.9% for crow 1 and 77.3% for crow 2 (Kruskal-Wallis tests, $p < 0.001$ for both short vs. long and long vs. short categorizations in both crows).

Both crows were slightly better in discriminating the control protocol compared with the standard protocol (paired t test, $p < 0.001$, Figures 1E and 1F). The mean performance of crow 1 with standard and control stimuli was 84.9% and 89.8%, respectively. Crow 2 had a mean performance of 84.0% with standard stimuli and 92.1% with control stimuli. However, the performance of both crows in each session was clearly above the 50% chance level with either stimulus set (all binomial tests for individual sessions and both crows $p < 0.01$).

Neuronal data

We recorded the single-cell activity of 449 NCL neurons (crow 1, 195 neurons; crow 2, 254 neurons) while the crows performed the length categorization task. Overall, 134 neurons (29.8% overall; 43% in crow 1 and 20% in crow 2) were found to be category selective in specific trial intervals and showed firing rate differences between the short vs. long categories (two-factor ANOVA, $p < 0.01$), but no differences within the two categories (Kruskal-Wallis tests, $p \geq 0.05$). In the sample phase, 65 neurons were category selective (Figure 2A), whereas 86 neurons were category selective during the delay phase (Figure 2B). The responses of four category-selective example neurons are shown in Figures 2C–2F. The neurons in Figures 2C and 2D increased their firing rates selectively to the line stimuli of the long category in the sample phase and during the delay, respectively. The other two example neurons responded selectively to category short in the early (Figure 2E) and later delay (Figure 2F), respectively.

The preferred category of a selective neuron was defined as the one eliciting the highest firing rate within the selective time window. In the sample phase, slightly more neurons preferred the short category (n = 41/65, binomial test, $p = 0.046$): in the delay, a similar number of 47 selective neurons preferred short, whereas 39 neurons preferred the long category (binomial test, $p = 0.45$)

Category-selective neurons robustly encoded the learned categories; their tuning fulfilled the hallmarks of categorical responses, i.e., a similar response to all members of the same category and a change in activity across the category boundary (Figures 2G and 2H). Both in the sample and the delay phase, a significant difference between the neurons' firing rates to the preferred vs. the non-preferred categories was observed (Wilcoxon signed-rank test, $p < 0.001$). No firing rate differences were found within the preferred and non-preferred categories for sample and delay (Friedman tests, $p > 0.05$), except for firing rates within the non-preferred category of sample-selective neurons (Friedman test, $p = 0.02$).

We calculated a category index to quantify the difference in the firing rates of the category-selective neurons (analogous to Freedman et al.[37,53]). We first derived the "within-category difference" (WCD) and "between-category difference" (BCD) from the neurons' firing rates (see STAR Methods). For the population of selective neurons, the BCD was significantly higher than the WCD, resulting in a shift of the data above the diagonal when plotted against each other (Figures 2I and 2J) (Wilcoxon signed-rank test, $p < 0.001$ for both sample [n = 65] and delay-selective neurons [n = 86]). The WCD and BCD were then used to calculate the category index—positive index values (max. +1) indicate higher firing rate differences for stimuli of different (across) categories, whereas negative values (min. −1) signify higher differences for stimuli of the same (within) category (Figures 2K and 2L). For both the sample- and delay-selective category neurons, the distributions were significantly shifted toward positive values with means of 0.36 and 0.45, respectively (both one-sample t tests, $p < 0.001$), indicating strong category coding of the selective

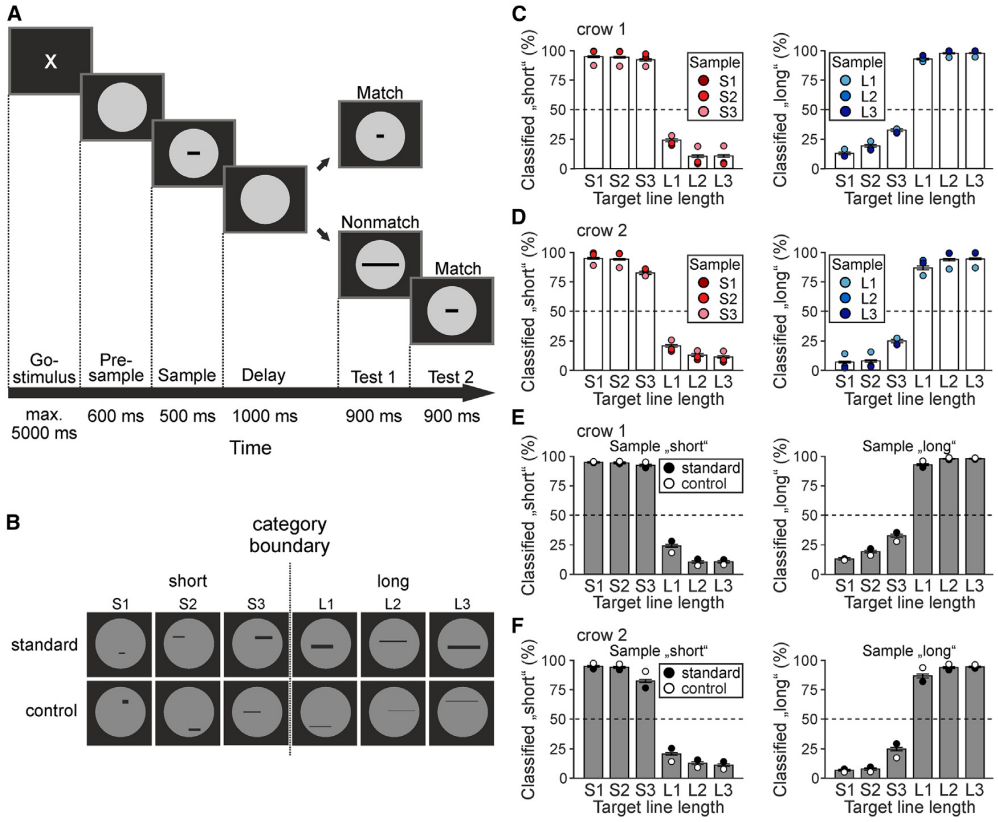

Figure 1. Task protocol, example stimuli, and behavioral performance in the two-category task

(A) Layout of the delayed match-to-category task with line-length stimuli. The crows had to respond whenever test 1 in 50% of the trials showed a line length that matched the short-vs.-long length category of the sample. In the other 50% of the trials, test 1 was a category "nonmatch"; here, the crow had to refrain from responding until the second test stimulus (test 2) was shown, which was always a category "match."

(B) Example stimulus displays of the two-category task. Two stimulus sets (standard and control) with six line lengths each were used. Category boundary divided the stimuli into short and long categories, with three line lengths each.

(C) Percent correct performance of crow 1 in the two-category task. Left: performance in trials with short sample stimuli. Right: performance in trials with long sample stimuli. The values depict the percentage of how often the crows correctly judged the length of either test 1 or test 2 as belonging to the same category as the line length of the sample stimulus. The circles indicate which exact line length was previously shown as the sample stimulus. Chance level is 50% (dashed lines). Error bars (very small) represent SEM across the sessions.

(D) Same as in (C) but for crow 2.

(E) Behavioral performance of crow 1 for the two different stimulus sets (standard and control) individually. Left: performance in trials with short sample stimuli. Right: performance in trials with long sample stimuli. Chance level is 50% (dashed lines). Error bars (very small) indicate SEM across the sessions.

(F) Same as in (E) but for crow 2.

neurons. Category indices had a tendency to be larger during the delay period (two-sample t test, $p = 0.067$).

Analysis of the population of category-selective neurons

To assess the activity of the category-selective neurons together across time, we transformed their activity into state space. Here, the activity of a population of neurons at every moment is represented as an n-dimensional vector in n-dimensional space. After dimensionality reduction to the three most informative dimensions (first three principle components [PCs]), the trajectories in three-dimensional space represent the time course of the neuronal activity to the different line lengths (see STAR Methods). Sample-selective and delay-selective category neurons were separately analyzed. Figures 3A and 3B depict the resulting activity trajectories across trial time in state space. While the absolute position of the color-coded trajectories representing the six line lengths is irrelevant, the distances between trajectories reveal differences in population activity. Visual inspection shows that similar line lengths are encoded in an orderly fashion by nearby trajectories. In addition, trajectories within a category seem to be closer, whereas trajectories across the two categories appear more distant.

We performed a cluster analysis to explore the potential clustering of population activity according to the categories. We calculated PC scores with average firing rates in the sample and delay period separately (see STAR Methods). The dispersion

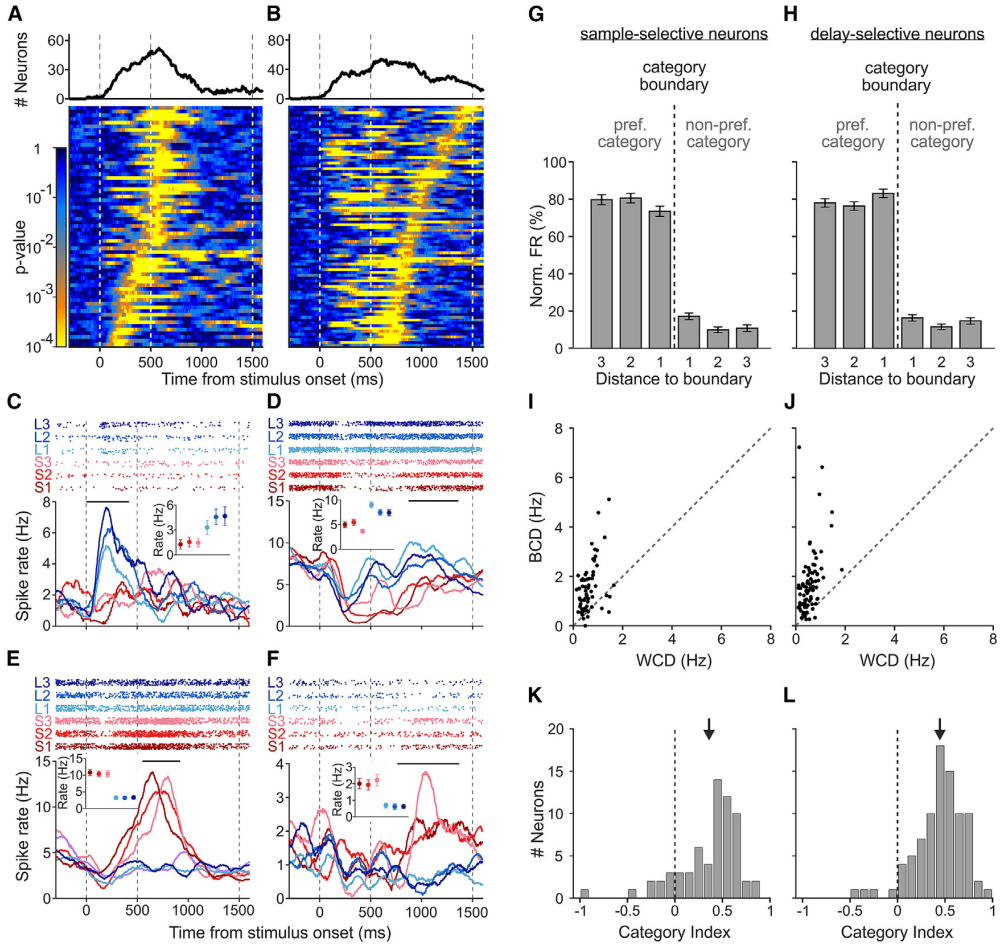

Figure 2. Single-neuron activity from NCL in the two-category task

(A and B) Pattern of task-selectivity of the neurons with a selective interval in the sample (A) and delay phase (B). Top: time-resolved histograms depicting the number of neurons for which factor "category" was significant at a given time point. Bottom: color-coded traces of the p values. Each line represents a neuron. Dashed lines separate the periods of a trial by indicating sample onset (at 0 ms), sample offset (at 500 ms), and end of delay (1,500 ms).

(C–F) Responses of four single neurons selective to category long in the sample (C) and delay phase (D), respectively, and selective to category short in the early delay (E) and later delay (F). Top panels depict dot-raster histograms (each line corresponds to a trial and each dot is an action potential). Bottom panels represent the corresponding averaged and smoothed (200 ms Gauss kernel, step size of 1 ms) spike-density functions. Each line shows the time course of the activity for the six different line lengths. Vertical dashed lines indicate sample onset, sample offset, and end of delay. The horizontal black line indicates the selective interval. Tuning function insets show the average firing rate to each line length during this interval (error bars indicate SEM across the trials).

(G and H) Average normalized activity of category-selective neurons in the sample (G) and delay phase (H) in response to the individual line lengths of their preferred and non-preferred category. The line lengths are arranged according to their distance from the category boundary. Error bars indicate SEM.

(I and J) Difference in firing rates in response to sample line lengths of the same (WCD) and different categories (BCD) for sample (I) and delay category-selective neurons (J).

(K and L) Frequency distribution of category indices for sample (K) and delay category-selective neurons (L). Arrows indicate respective means.

of the PC scores (only the first two PCs) for each trial (n = 180) in PC-space is shown in Figures 3C and 3D. We first determined the optimal number of clusters for the datasets by applying two measures: the Caliński-Harabasz index (also termed "variance ratio criterion [VRC]"),[54] and the "gap criterion" that determines the most dramatic decrease in error measurement (the "elbow" or "gap") of different cluster numbers (see STAR Methods).[55] In the sample period, the Caliński-Harabasz index (which is only defined for two or more clusters and thus less reliable) indicated two as the optimal cluster number, whereas the gap value indicated only one cluster as an optimal description of the population activity (Figure 3E). However, in the delay period, both measures indicated two clusters as the optimal cluster number (Figure 3F).

We then applied unsupervised k-means clustering to partition all trials in state space (n = 180) into the previously determined

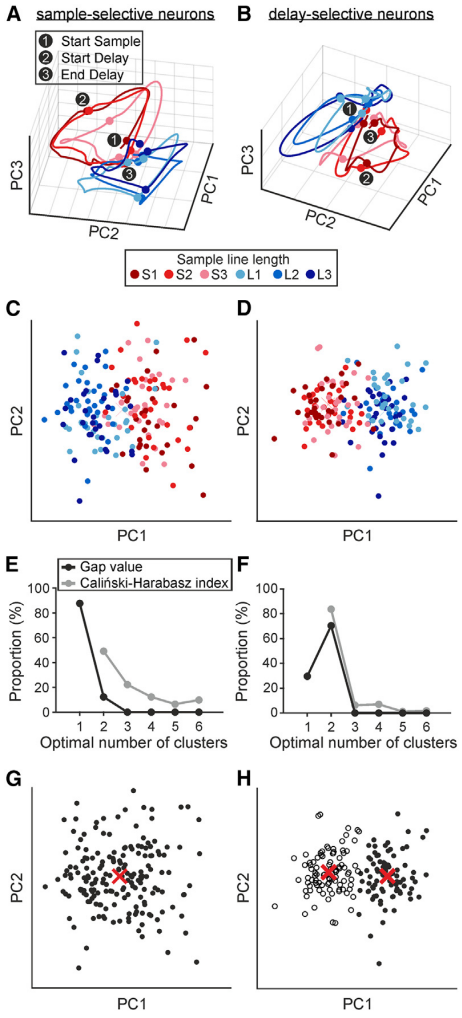

Figure 3. State space analysis of the selective neurons of the two-category task

(A and B) Time course of neuronal activity to the different line lengths throughout a trial (1, start sample phase; 2, start delay; 3, end of delay) of neurons that were category selective in the sample (A) and delay phase (B).

(C and D) Dispersion of the PC scores of an example clustering repetition during the sample (C) and delay phase (D). One dot corresponds to one trial, color-coded by the different sample line lengths.

(E and F) Proportion of the optimal number of clusters based on gap value and Caliński-Harabasz index, respectively, in the sample (E) and delay phase (F).

(G and H) Cluster assignment based on gap value of the same trials as in (C) and (D), respectively. The optimal number of clusters was "one" in the sample phase (G) and "two" in the delay phase (H). Red crosses indicate the position of the cluster's centroids.

optimal number of clusters.[56] In the sample period, the indifferent data comprised a single cluster (Figure 3G). In the delay period, however, the clustering algorithm detected one cluster for each of the two (short vs. long) length categories (Figure 3H). However, with trial progression, activity in state space encodes the relevant two length categories by two clusters that border between the length categories.

Analysis of the entire neuron population

In the next step, we explored the category coding capability of the entire population of recorded neurons (n = 348), irrespective of selectivity. We focused on the last 600 ms of the delay period in which the crows particularly relied on category information to solve the task. First, we calculated a correlation matrix to compare the responses of the neurons with pairs of stimuli (Figure 4A). A correlation coefficient was calculated for each stimulus combination, and its value is depicted as a color-coded tile in the correlation matrix. The emerging correlation pattern shows that the responses of all neurons were more similar to within-category stimuli than to across-category stimuli. The mean coefficient for correlations for within-category stimuli was 0.76 and thus higher compared with the mean coefficient of 0.63 for across-category stimuli (two-sample t test, $p < 0.001$) (Figure 4B).

To explore the behavioral relevance of population activity for the crows' categorization performance, we calculated the correlation coefficients for suitable neurons also in error trials in addition to correct trials over the last 600 ms of the delay period (Figures 4C and 4D). The correlation coefficients in correct trials differed significantly for this subset of neurons, with means of 0.77 and 0.62 for stimuli of the same and different categories, respectively (two-sample t test, $p < 0.001$). In error trials, however, no difference between within-category correlation coefficients (mean = 0.41) and between-category correlation coefficients (mean = 0.39) was found (two-sample t test, $p = 0.70$) (Figure 4E). This indicates that the activity differences between categories that are lacking in error trials are behaviorally relevant for the crows to group the sample stimuli into the learned categories during correct trials.

Next, we used a population decoding approach to explore categorical information contained in the neuronal responses. We trained a support vector machine (SVM) classifier with the firing rates of the neurons within the last 600 ms of the delay.[57] The classification performance was then tested with a subset of these firing rates which were not used for training (Figure 4F). The classifier grouped the firing rates with a high performance of 91.2% (±0.9% SEM) into the correct categories. Additionally, we trained an SVM classifier 3 times on different pairs of training stimuli to test whether the firing to each of the six individual stimuli was predictive of the short vs. long categorization. For each classifier training, we used the firing rates to one stimulus of each category (S1/L3, S2/L2, and S3/L1, respectively) and then predicted the category of the remaining four stimuli. The classifier was able to predict the correct category at a mean performance of 77.9% (±3.2% SEM) (Figure 4G). All training sets resulted in similarly high classifier performance without performance differences between the tested cross-category line pairs (two-factor ANOVA, $p = 0.58$; mean classification performance with S1/L3 as the training set, 76.0%; with S2/L2, 83.0%; with S3/L1, 74.8%). Importantly, there was no difference between the performance of stimuli within the short and the long category (two-factor ANOVA, $p = 0.52$; mean prediction performance for stimuli of the short category: 75.7% and long category: 80.2%). These decoding results show that the neurons respond in a similar manner to all stimuli of the same category but differently to members of the other category, thus allowing a classifier to predict category membership.

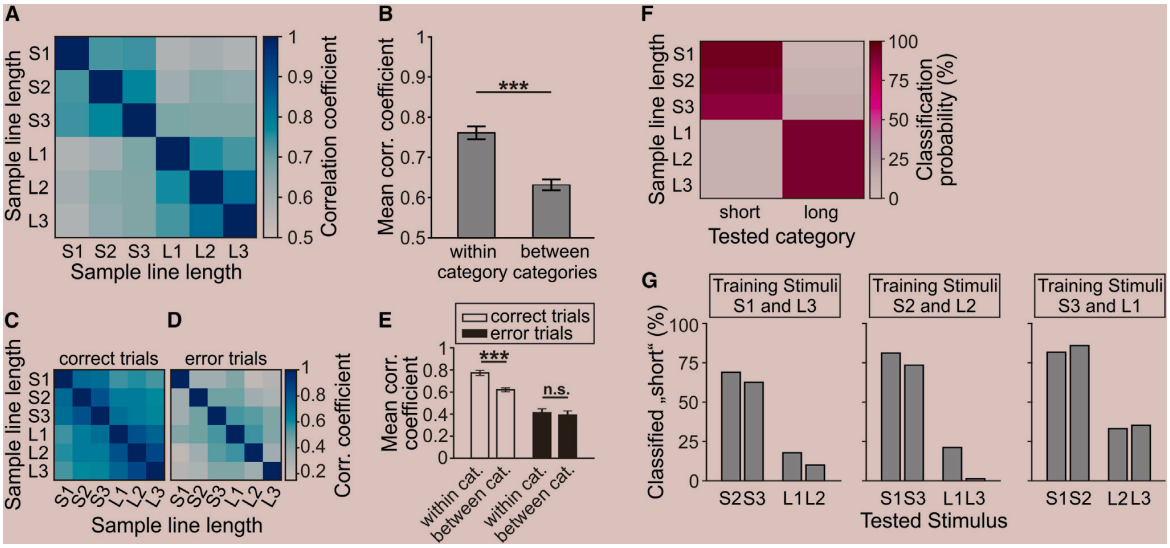

Figure 4. Correlated activity to pairs of stimuli and classification probability of an SVM classifier for the entire neuronal population in the two-category task

(A) Correlation matrix to pairs of stimuli comparing the neuronal activity during the last 600 ms of the delay. Each tile represents the correlation coefficient via color code. Darker colors indicate higher correlation. The tiles along the diagonal represent the maximum correlation when comparing stimuli with themselves (r = 1.0).

(B) Mean correlation coefficient across all comparisons of stimuli within the same category and of different categories, respectively. Error bars represent SEM. ***: $p < 0.001$.

(C and D) Correlation coefficients in correct (C) and error trials (D) for a subset of the neuronal population for which error trials could be analyzed.

(E) Mean correlation coefficients across comparisons of stimuli within the same category and of different categories in correct trials (left two bars) and error trials (right two bars). Error bars represent SEM. ***: $p < 0.001$.

(F) Performance of an SVM classifier classifying the category of the sample stimulus after being trained on the firing rates of the entire neuronal population during the last 600 ms of the delay.

(G) SVM classifier predictive performance to novel stimuli after classifier training on the firing rates to two other stimuli. Data for classifier training with S1 and L3 (left), S2 and L2 (middle), and S3 and L1 (right). Columns represent the proportion of how often a stimulus was assigned to the short category.

Behavior in the three-category task

After collecting data in the two-category task, we retrained crow 1 on a three-category task to explore learning-related categorization changes. We used the same line-length stimuli but applied two category boundaries that resulted in the three length categories short, medium, and long (Figure 5A). The crow was able to learn the new categories and performed above the 50% chance level in each session (all binomial tests, $p < 0.001$). The mean correct performance across all sessions was 83.1% (±3.7% SEM, n = 58 sessions).

The crow showed similarly high performances for either stimulus of each category and a sharp drop-off across the two category boundaries (Figure 5B). As with the two-category task, the crow performed best when the sample stimulus was S1 (95.0% ± 0.5% SEM, Figure 5B, left). However, performance was also high for the medium category which is the most difficult category because both within-category stimuli are adjacent to a category boundary (M1, 81.3% ± 0.9% SEM; M2, 75.1% ± 1.0% SEM; Figure 5B, middle). The mean performance with standard and control stimuli was 81.4% (±0.7% SEM) and 85.2% (±0.7% SEM), respectively, and thus slightly better with stimuli of the control set (paired t test, $p < 0.001$, Figure 5C). However, the crow's performance in each session was well above chance level with both stimulus sets (all binomial tests, $p < 0.001$).

Neuronal data in the three-category task

We recorded 336 single neurons while crow 1 was performing the three-category task. Of these, 128 neurons (38.1%) were category selective (47 neurons in the sample phase and 93 neurons during the delay). Three category-selective neurons are shown in Figures 5D–5F. The sample-selective neuron in Figure 5D was tuned to the long category. The other two delay-selective example neurons preferred the medium category in the middle of the delay (Figure 5E) and the long category toward the end of the delay (Figure 5F), respectively. In the sample phase, 14 neurons preferred a stimulus of the short category, 11 of the medium, and 22 of the long category. During the delay, 17 preferred short, 53 preferred medium, and 23 preferred long.

Analogous to the analysis of the two-category data, we assess the activity of all selective neurons in the three-category task together in state space (Figures 6A and 6B). In addition to the orderly representation of adjacent line lengths, trajectories within one of the categories seem to be closer, whereas trajectories across categories appear more distant. We again performed cluster analysis with PC scores (n = 180 trials) and separately for the sample and delay periods (Figures 6C and 6D).

In the sample period, the Caliński-Harabasz index indicated three as the optimal cluster number, whereas the gap value indicated only two clusters as an optimal description of population

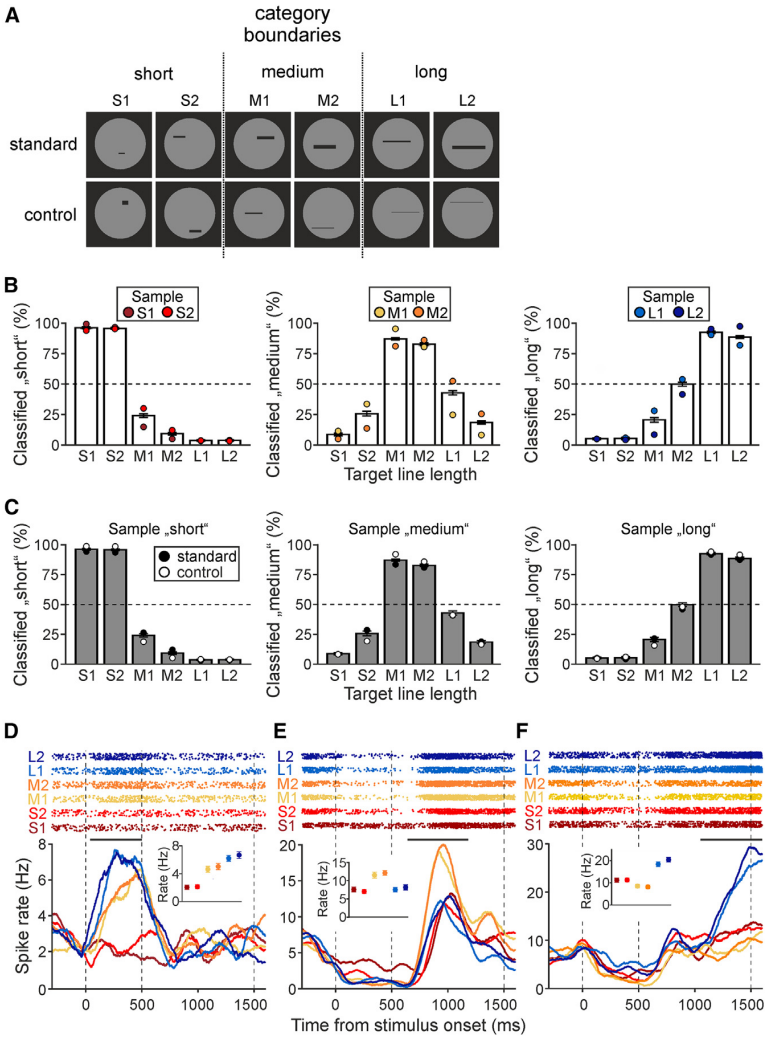

Figure 5. Example stimuli, behavioral performance, and single-neuron activity in the three-category task

(A) Example stimulus displays of the three-category task. Two different stimulus sets (standard and control) with six line lengths each were used. The category boundaries divided the stimuli into three categories (short, medium, and long), with two line lengths each.

(B) Behavioral performance of crow 1 in the three-category task. Left to right: performance in trials with short, medium, and long sample stimuli, respectively. Circles indicate which line length was shown as the sample stimulus. Dashed lines represent chance. Error bars (very small) represent SEM across the sessions.

(C) Behavioral performance of crow 1 for the two different stimulus sets (standard and control) individually. Left to right: performance in trials with short, medium, and long sample stimuli, respectively. Layout as in (B).

(D) Category-selective neuron encoding the three categories in the sample phase and preferring category long. Layout as in Figures 2C–2F.

(E and F) Example category-selective neurons during the delay, preferring the medium (E) and long categories (F), respectively. Layout as in Figures 2C–2F.

activity (Figure 6E). However, in the delay period, both measures indicated three as the optimal cluster number (Figure 6F). In the sample period, k-means clustering partitioned the trials into the previously determined two optimal clusters which mainly consisted of the first shortest vs. the three longest line stimuli (Figure 6G). In the delay period, however, the clustering algorithm detected three clusters correlating with the line lengths of the short, medium, and long categories (Figure 6H). Thus, state space activity later in the delay period encodes the relevant three length categories by three clusters that border between the trained length categories.

As before, we analyzed the category coding of the entire population of recorded neurons (n = 278) and used again the activity in the last 600 ms of the delay period to calculate a correlation matrix. After retraining crow 1, the correlation pattern now reflected the new three categories (Figure 7A). The difference between the mean correlation coefficient for stimuli within the same category (0.80) and for correlations of stimuli across categories (0.59) was significant (Figure 7B) (two-sample t test, $p < 0.001$). By contrast, no differences in correlation coefficients were observed for stimuli that belonged to adjacent categories (short vs. medium and medium vs. long; mean = 0.57) or had a greater distance (short vs. long; mean = 0.62) (two-sample t test, $p = 0.06$).

We tested whether the neuronal population recorded during the three-category task may still encode the now invalid two categories of the original task. The mean correlation coefficients for stimuli within the original categories of the two-category task compared with stimuli between the original two categories were 0.65 and 0.61, respectively, and were indifferent (two-sample t test, $p = 0.44$). This indicates that the population of neurons no longer encoded the categories of the two-category task. As a control, we explored three-category coding in the original two-category task and calculated the correlation analysis with the categories of the three-category task for the data recorded during the two-category task (see Figure 4A). Here as well, the mean correlation coefficients for stimuli within the same category (0.74) and those between categories (0.67) were indifferent (two-sample t test, $p = 0.12$). Thus, the correlation differences within and between categories of the three-category task were not a chance event but resulted from retraining with the new categories.

A subset of the neuronal population (n = 120 suitable neurons; see STAR Methods) was used to analyze the correlation coefficients in error trials (Figures 7C and 7D). Although a higher correlation coefficient for stimuli within the same category than for stimuli across categories (0.82 and 0.56, respectively) was

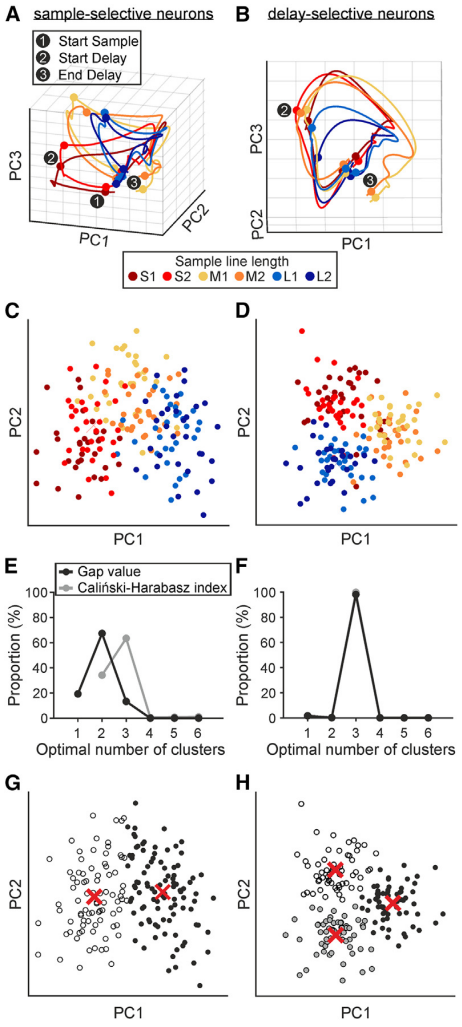

Figure 6. State space analysis of the selective neurons of the three-category task

(A and B) Course of neuronal activity in response to the different line lengths throughout a trial (1, start sample phase; 2, start delay; 3, end of delay) for neurons that were category selective in the sample phase (A) and during the delay (B).

(C and D) Dispersion of the PC scores of an example clustering repetition during the sample period (C) and during the delay (D). One dot corresponds to one trial, color-coded by the different sample line lengths.

(E and F) Proportion of the optimal number of clusters based on gap value and Caliński-Harabasz index, respectively, in the sample phase (E) and during the delay (F).

(G and H) Cluster assignment based on gap value of the same trials as in (C) and (D), respectively. Here, the optimal number of clusters was two in the sample phase (G) and three in the delay (H). Red crosses indicate the position of the cluster's centroids.

observed in correct trials (two-sample t test, $p < 0.001$, Figure 7E), in error trials, no difference between the correlation coefficients for stimuli of the same category and stimuli across categories was detected (both 0.32, two-sample t test, $p = 0.99$). This again indicates that category-specific signals in the three-category task had vanished during error trials, supporting the behavioral relevance of the neurons' responses.

Finally, we employed decoding population analyses irrespective of single neurons' category selectivity. We again trained an SVM classifier using the firing rates of the last 600 ms of the delay and tested its classification performance with a subset of firing rates that were not used for training (Figure 7F). The classifier grouped the firing rates correctly into the three categories with a high probability of 90.1% (±1.5% SEM). This indicates a robust representation of the three length categories by the random population of NCL neurons.

DISCUSSION

We report that crows efficiently learned to apply a matching to category rule based on short or long line length. We report three major findings from recordings during task performance. First, a substantial proportion of neurons encoded the category information by showing large activity differences between length categories but similar responses to stimuli within each length category. Second, after the retraining of a crow and testing with new and more length categories (short, medium, and long), NCL neuron activity had flexibly changed to now reflect these new categories. Our data show malleable categorization capability mediated by the flexible networks of the crow NCL that are reminiscent of findings in the PFC of monkeys.

Temporal dynamics of behaviorally relevant category activity with trial time

The task design allowed us to compare length category selectivity during the visual encoding phase (sample phase) and during memorization (delay phase). During the two-category task, more category-selective neurons, with stronger selectivity, were observed during the delay compared with the sample period. Moreover, behaviorally relevant activity clusters (derived from state space) based on the population of category-selective neurons only emerged during the delay period but were largely absent during the sample period in both two-category and three-category tasks. In addition, activity differences between length categories (as measured by correlation measures) collapsed during the delay period in error trials in both two-category and three-category tasks. These results suggest that category-indifferent neural responses rendered the crows' error-proneness.

Collectively, our data argue that the activity of NCL neurons is relevant for the crows' categorization performance, confirming previous findings during numerical categorization.[47,48] Moreover, the observed time course of category selectivity indicates that the initial sensory and largely category-void encoding of length stimuli became dynamically restructured along trial time. Direct sensory length information in conjunction with conceptual information (i.e., short, medium, and long categories) retrieved from long-term memory sculptured NCL activity until the crows have access to categorical representations at the end of the delay period when they need this data to solve the task.

Mechanism of categorization

Mechanistically, the emergence and shaping of categorical tuning could be implemented via broad inhibitory mechanisms.[58] To pin

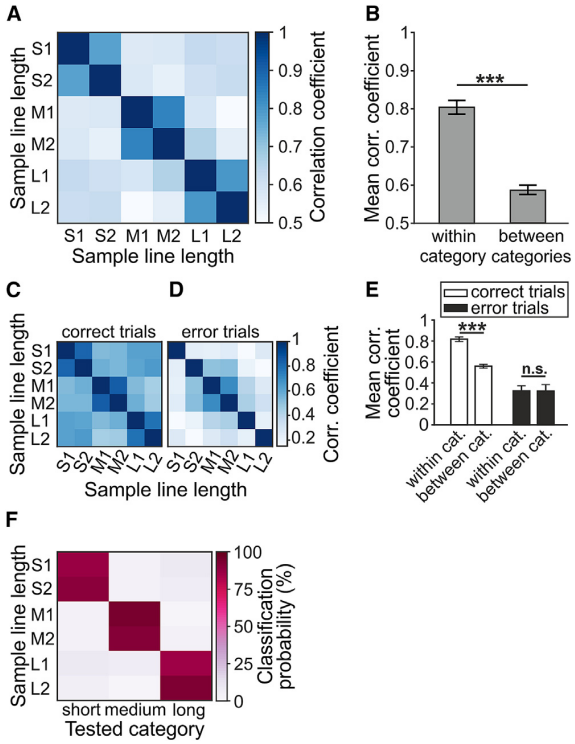

Figure 7. Correlated activity to pairs of stimuli and classification probability of an SVM classifier for the entire neuronal population in the three-category task

(A) Correlation matrix comparing the neuronal activity during the last 600 ms of the delay. Layout as in Figure 4A.

(B) Mean correlation coefficients across all comparisons of stimuli within the same category and of different categories, respectively. Error bars represent SEM. ***: $p < 0.001$.

(C and D) Correlation coefficients in correct (C) and error trials (D) for a subset of the neuronal population for which error trials could be analyzed.

(E) Mean correlation coefficients across comparisons of stimuli within and between categories in correct (left columns) and error trials (right columns). Error bars represent SEM. ***: $p < 0.001$.

(F) Performance of SVM classifier trained on firing rates of the entire neuron population during the last 600 ms of the delay. Layout as in Figure 4F.

down inhibitory mechanisms, the major pallial cell types, putative excitatory projection cells and inhibitory interneurons, have been identified by means of waveform analyses of intra- and extracellularly recorded action potentials.[59–62] Waveform analyses and segregation of putative excitatory projection and inhibitory interneurons were recently also accomplished in NCL neurons of crows discriminating numerosities. It turned out that putative inhibitory interneurons showed stronger stimulus-evoked responses, shorter response latencies, and broader numerosity tuning compared with putative projection neurons.[24] In addition, nearby and functionally coupled putative excitatory projection neurons were synchronously excited and exhibited similar numerosity tuning, whereas coupled putative inhibitory interneurons and projection neurons inhibited each other's firing and showed inverse tuning relative to each other.[24] These data suggest an inhibitory feedforward mechanism for the shaping of neurons tuned to numerical categories in the crow NCL.[24] Such a microcircuit ensures that only projection cells that respond to the correct category remain active and control the animal's response.

Category selectivity arising through reinforcement learning

The length categories applied in the current task—first, short vs. long, and later, short, medium, and long—had no congenital origin and needed to be learned by the crows over time as the result of trial-and-error reinforcement learning. Reinforcement learning based on reward can refine functional connectivity between neurons[63] and typically relies on dopamine signals[64,65]—reward prediction error signals arising from the dopamine system modulate reward-dependent plasticity in primates.[66] Similar processes may be at work in birds learning to categorize, as reward prediction errors have also been observed in the avian NCL, a pallial brain area that is characterized by strong dopaminergic innervation.[33,35,67] According to a cortical circuit model designed for neuronal category learning, weak but systematic correlations between trial-to-trial fluctuations of the firing rates and the accompanying reward after appropriate behavioral choices generate neurons that gradually become category selective.[68] In this model, initially nonselective neurons that show fluctuations that correlate with behavioral outcome developed categorical tuning. Therefore, when a crow learns to respond appropriately to length categories in order to receive a reward, such a mechanism might suffice to produce category-selective NCL neurons from originally untuned neurons. Alternatively, the NCL may contain a special set of malleable category-tuned neurons that change their boundaries with experience. In a previous study, we reported that association learning exclusively recruited NCL neurons that already represented previously established associations.[69] Translated to categories, learning could cause the same pool of neurons to respond to new category boundaries applied to the same length stimulus space.

Category representations in crow NCL vs. primate PFC

The avian NCL is often said to be a functional equivalent of the primate PFC. A comparison of the current data in the crow NCL adds to this functional resemblance in the realm of learned categorization. In primates, behaviorally relevant representations of learned categories have been studied extensively in the PFC using delayed match-to-category tasks. In a seminal series of experiments, macaques were trained to categorize morphed visual stimuli into arbitrary cat and dog categories.[37–40,53,70] As expected for category selectivity, a large proportion of PFC neurons encoded category information by exhibiting significant activity differences between cat and dog categories but similar responses to stimuli within each category.[37–40,53,70] Moreover, although the monkeys learned new category boundaries within the same stimulus space, PFC neurons changed selectivity to now encode the new category boundary, indicating the malleability of the PFC in representing acquired categories.[37–40,53,70]

Beyond perceptual categories (such as cats and dogs), the primate PFC is also equipped to represent more abstract learned spatial categories comparable to those tested here in crows. In the past, representations of abstract magnitude, such as the

absolute[71,72] and relative line length,[73,74] the absolute[75] and relative spatial distance,[76,77] or numerical quantity[78,79] have been reported in the macaque PFC. In one study, monkeys had to learn to classify spatial proportions, i.e., the relation between the variable lengths of two horizontal lines, with proportions ranging from 1:4 and 2:4 to 3:4 and 4:4.[80] Here, PFC neurons showed categorical proportion tuning to the four different proportion categories, very similar to the three length categories short, medium, and long reported here in crows.

These similarities in the flexibility of telencephalic associative brain areas to represent abstract learned categories are remarkable in the face of independent evolution of these brain areas in mammalian and avian lineages.[81] Compared with the mammalian neocortex, the avian telencephalic integration centers originate from different pallial territories during embryology,[82] show distinct neural architectures,[83] and have evolved classes of excitatory and inhibitory pallial neurons that have no counterpart in the mammalian neocortex.[84–86] Despite all this independent brain evolution, crows and monkeys seem to be equipped with equivalent neuronal circuits that can flexibly represent abstract learned magnitude categories.[19,87]

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS

A.N. and L.W. designed the study, interpreted the data, and wrote the manuscript. L.W. performed experiments and analyzed the data. All authors gave final approval for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Corvus corone	University of Tübingen, Institute of Neurobiology	crow 1, crow 2
Software and algorithms		
NIMH Cortex	National Institute of Mental Health	c595; https://www.nimh.nih.gov/labs-at-nimh/research-areas/clinics-and-labs/ln/shn/software-projects.shtml
MAP Data Acquisition System	Plexon	https://plexon.com/
MATLAB R2019a	MathWorks	https://www.mathworks.com
Other		
Dental Cement	Heraeus	Paladur, ISO 20795, CE 0197
Microdrives	Animal Physiology Unit	Custom fabrication
Electrodes	Alpha Omega LTD	Cat.#: 366-130620-00 www.alphaomega-eng.com

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Andreas Nieder (andreas.nieder@uni-tuebingen.de).

Materials availability

This study did not generate new unique reagents.

Data and code availability

All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to re-analyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Subjects

Two hand-raised adult male carrion crows (*Corvus corone*) from the institute's breeding facility were used. The crows were 3 and 6 years old. They were housed in an indoor aviary in social groups. During the experiment, the crows were on a controlled feeding protocol and received their daily amount of food as reward during training and recording or, if necessary, after the sessions. Water was available *ad libitum* during the experiments and in the aviary. All procedures were carried out according to the guidelines for animal experimentation and approved by the responsible national authorities, the Regierungspräsidium Tübingen, Germany.

METHOD DETAILS

Apparatus

The experiment was conducted in a darkened operant conditioning chamber. The crows were placed on a perch in front of a 15" touchscreen monitor (ART development MT1599-BS and ART development PS-150, respectively). Viewing distance to the screen was 14 cm. The touchscreen was used only for stimulus presentation, as the crows responded by head movements.

Behavior and response of the crows were controlled by an infrared reflexive light system which was located above the crows and registered the position of a reflector foil attached on top of the crows' head. The crows initiated trials by keeping their heads still in the center position in front of the touchscreen monitor and were required to keep the head within this position throughout the trial until the target stimulus appeared.

The crows reported the detection of the target stimulus by briefly moving their heads ('nodding'), which was again automatically detected by the infrared reflexive light system. With every correct answer, a food reward (either birdseed pellets or mealworms

(*Tenebrio molitor* larvae)) was given by the briefly illuminated feeder below the touchscreen monitor. Auditory feedback was provided by speakers (Lasmex S-03) located behind the touchscreen monitor. We used the CORTEX system (National Institute of Mental Health) to run the experiment and collect behavioral data.

Behavioral protocol

The crows were trained to group horizontal line stimuli into learned categories according to their length (Figure 1A). The crows initiated a trial by positioning their heads facing the screen whenever the go-stimulus (small white cross, 2x2 dva (degree of visual angle)) was shown. A click sound indicated that the correct position had been entered and the go-stimulus turned briefly (for 60ms) into a circle before it vanished. This head position had to be maintained throughout the trial until the test phase. Premature head movements aborted the ongoing trial which was then discarded.

After a 600 ms pre-sample phase in which only the grey background circle was shown, the sample stimulus was presented for 500 ms. Then the screen returned to the grey background circle for a delay of 1000 ms. In the subsequent test phase, the first test stimulus (Test 1) appeared for max. 900 ms. In 50% of the trials, Test 1 displayed a line length that belonged to the same category as the sample stimulus (i.e. ''match''). In the other half of the trials, Test 1 was not member of the same category as the sample stimulus (i.e. ''nonmatch''). The chance level of Test 1 or Test 2 being a match was therefore 50%. The crow indicated a category ''match'' by instantaneously nodding, i.e. moving its head out of the monitored center position.

A ''nonmatch'' stimulus required the crow to maintain head position and to refrain from responding until the subsequent second test stimulus (Test 2) appeared which always belonged to the same category as the sample (a ''match''). A correct response to a ''match'' stimulus (either Test 1 or Test 2) led to a reward for the crow. A response to a ''nonmatch'' stimulus or no response to either Test 1 or Test 2 aborted the trial and was considered as error trial and not rewarded. Within each session, all behaviorally relevant parameters (i.e. sample line length, stimulus sets and match/nonmatch trials) were balanced and pseudo-randomly interleaved.

Stimuli

The line length stimuli were generated using MATLAB software. They consisted of a horizontal black line at random position within a grey background circle (Figure 1B). Six different line lengths were used that were assigned to either two (first experiment) or three different length categories (second experiment). The lengths were consecutive multiples of lengths of 2.6 dva ranging from 3.3 dva (shortest line) to 16.3 dva (longest line).

To ensure that the crows categorized length rather than the area or thickness of the lines, we used two interleaved sets of stimuli in each session, a standard stimulus protocol and a control protocol. In the standard protocol, the thickness of the lines varied randomly between 0.4 and 2.0 dva. In the control protocol, the black area of each line was kept constant to 6.5 dva^2 across the different lengths, with the thickness of the shortest line being always 2 dva and thickness of the longest line always 0.4 dva. In addition, the sample and the test images within a trial were never identical. New stimulus sets were generated for each session to prevent the crows from memorizing visual patterns.

First, we trained both crows on the two-category task (Experiment 1). For that, one category boundary divided the six different line lengths into two groups of three line lengths each. The ''short'' category included the lengths S1, S2 and S3; the ''long'' category included L1, L2 and L3. Thereafter, we retrained crow 1 on the three length categories ''short'', ''medium'', and ''long'' (Experiment 2). To that aim, we divided the line lengths into three categories of two line lengths each. The absolute lengths of the lines remained unaffected, i.e. the physical appearance of the stimuli stayed the same, only the category membership changed. The ''short'' category still included the lengths S1 and S2, and the ''long'' category still contained the lengths L2 and L3 (now renamed as L1 and L2, respectively). The former lengths S3 and L1 constituted the new ''medium'' category and in this context were renamed as M1 and M2, respectively.

Surgery and neurophysiological recordings

The surgeries were performed while the animal was under general anesthesia with a mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg). The animals were placed in a stereotaxic holder. We targeted the dorsal part of the *nidopallium caudolaterale* (NCLd)[33,35,36] by performing a craniotomy at 5 mm anterior-posterior and 13 mm medio-lateral on the right hemisphere. Two manual micro drives containing four electrodes each (2 MΩ, Alpha Omega Co.) and a miniature connector for the head stage were implanted. After the surgery, the crows received an analgesic. A small holder for attaching the reflector of the light barrier and head-tracking system, respectively, had been already implanted under the same conditions.

Each recording session started with adjusting the electrodes until a proper neuronal signal (of at least 3:1 signal to noise) was detected on at least one channel (see also Figures 4A and 4B in Veit and Nieder,[42] for an example recording trace). Neurons were not preselected in the involvement of the task. Signal amplification, filtering, and digitizing of spike waveforms was performed using the Plexon MAP system (Plexon Inc., Dallas, Texas). Spectral filtering of recordings was accomplished by a combined preamplifier filter (150 Hz–8kHz, 1 pole low-cut, 3 pole high-cut) and main filter (250 Hz, 2-pole, low-cut filter). Amplitude amplifications were set individually for different channels in the range of ca. 20,000x gain. Spike waveforms were sampled at a frequency of 40 kHz (one entry every 25 μs) for a duration of 800 μs. Plexon's offline Sorter was used to manually offline sort spikes into single-unit waveforms by applying mainly principal component analysis. We recorded 52 sessions in crow 1 and 55 sessions in crow 2 performing the two-category task, and 58 sessions in crow 1 performing the three-category task.

QUANTIFICATION AND STATISTICAL ANALYSIS

Behavioral analysis

The behavioral performance was measured as the percentage correct categorization of the sample line lengths, i.e. of how often the crows correctly judged that the line lengths of the test stimuli (either Test 1 or Test 2) belonged to the same category as the length of the sample stimulus. For each session we used a binomial test to verify that the ratio of correct answers was above the 50% chance level (for both stimulus sets separately and combined).

Neuronal analysis

Analyses of category-selective neurons

For the neuronal analyses, we included all neurons which had an average firing rate of at least 1 Hz during the overall trial and were recorded for at least 10 correct trials for each sample line length. First, we identified category-selective neurons. To that aim, we analyzed the activity of the neurons in sliding windows of 200 ms length which were advanced by 10 ms steps, starting at sample onset and ending 100 ms after delay offset (to account for the neurons' response latency). In each window, we performed a combination of two statistical tests on the neurons' firing rates to determine category-selective neurons: First, we calculated a two-factor ANOVA with category and stimulus protocol as main factors (criterion $P < 0.01$) to determine across-category selectivity. Neurons were selected that showed a significant main effect for category but no effect for main factor protocol or interaction between main factors. Second, we additionally calculated Kruskal-Wallis tests to explore differential activity to within-category line length. A category selective neuron was supposed to show no response differences across stimuli within each category (criterion $P \geq 0.05$). Neurons recorded in the two-category task were tested with two Kruskal-Wallis tests (for category "short": neuronal responses to sample stimuli S1 vs. S2 vs. S3, and for category "long": sample stimuli L1 vs. L2 vs. L3). Neurons recorded in the three-category task were tested with three Kruskal-Wallis testes ("short": neuronal responses to sample stimuli S1 vs. S2, "medium": sample stimuli M1 vs. M2, "long": sample stimuli L1 vs. L2). If a neuron fulfilled all of these criteria, i.e., was selective to category in the ANOVA but unselective in the Kruskal-Wallis tests over at least 11 consecutive windows (i.e. 300 ms in total), it was termed 'category-selective'.

A category selective interval was assigned to the sample period if it started no later than 100 ms after sample offset. Later occurring selective intervals were assigned to the delay period. If a neuron had more than one selective interval in the sample or delay period, respectively, only the one with the smallest *P*-value for factor category according to the ANOVA was used for later analyses. The preferred length category within a selective interval was defined as the category which contained the stimulus eliciting the highest mean firing rate. To calculate the average neuronal activity of the category-selective neurons within the selective intervals (separately for the sample and delay phase), each neuron's mean firing rates to the six different line lengths were normalized by setting the highest firing rate to 100% and the lowest to 0%. These were then arranged according to their distance from the category boundary and averaged across all neurons. Due to this definition of category selectivity, the preferred category is expected to be encoded by normalized firing rates that are larger than 50% and maximally 100% (and vice versa for the non-preferred category). However, the normalized firing rates to individual line length stimuli within a category are not part of the definition, which is why this measure and the derived values are suitable to explore neuronal encoding similarities to individual stimuli within categories and coding differences to stimuli between categories, particularly at the category boundary.

We calculated a category index from the average firing rates of the category-selective neurons to the six different sample line lengths analogous to Freedman et al.[37,53] The "between category difference" (BCD) was defined as the absolute difference between the average firing rate of a neuron to the sample stimuli adjacent to the category boundary (i.e. S3 vs. L1). For the "within category difference" (WCD), we calculated the firing rate differences between all neighboring sample stimuli (to keep the distance between the compared stimuli constant) that belong to the same category (i.e. S1 vs. S2, S2 vs. S3, L1 vs. L2 and L2 vs. L3) and then took the mean of these. From these firing rate differences, we calculated the category index by subtracting the WCD from BCD and dividing it by their sum:

$$\text{Catergory index} = (\text{BCD} - \text{WCD})/(\text{BCD} + \text{WCD}).$$

It resulted in values between -1 and 1 with positive values indicating a higher difference between the firing rates to two sample stimuli of different categories than between stimuli within the same category. Shifts of the category index distributions relative to value 0 were tested with a one-sample *t*-test.

We used principle component analysis (PCA), to investigate how the activity of the category-selective neurons evolved during the course of a trial. For the later purpose of clustering, we included all category-selective neurons with at least 30 correct trials for each line length (two-category task: 54 out of 65 sample-selective neurons and 80 of 86 delay-selective neurons; three-category task: 41 out of 47 sample-selective neurons and 88 out of 93 delay-selective neurons). The neuronal activity in response to a certain stimulus at a certain time point is represented as a *n*-dimensional vector in *n*-dimensional space, with each dimension corresponding to one single neuron.

We used PCA to reduce the dimensionality of the population activity while capturing most of the information. For that, the neuronal data for each trial was smoothed by a 200 ms Gauss kernel with a step size of 1 ms, and the mean firing rate to each line length was calculated in bins of 100 ms (advanced in steps of 10 ms) and then neuron-wise *z*-scored. From this data, we created a population of pseudo-simultaneously recorded neurons. We calculated the PC scores using the implemented *pca* function of MATLAB. To

illustrate the trajectories of the change in neuronal activity, we used the first three principle components which formed a three-dimensional subspace. These three principle components explained 47.2% of the neuronal covariance in the sample period, and 40.6% of the covariance in the delay period of the two-category task. In the three-category task, the first three principle components explained 58.0% of the neuronal covariance of the neurons which were category-selective in the sample phase and 54.6% for the neurons which were selective during the delay.

In a next step, we analyzed the sample and delay periods separately. As before, the neuronal activity was first smoothed by a 200 ms Gauss kernel across the entire trial. Then, we calculated the mean firing rate in each trial across a 600 ms time window starting at sample onset and reaching 100 ms into the delay for the neurons which were category-selective in the sample phase. For the neurons that were selective during the delay, we averaged the firing rate across the last 900 ms of the delay. Then, we randomly drew the firing rates of 30 trials for each sample line length within the given analysis interval, z-scored these neuron-wise and calculated the PC scores.

To evaluate the optimal number of clusters, we applied the unsupervised *k-means* clustering algorithm using the first two principle components. We used two different criteria, the gap value and the Calinski-Harabasz index.[54,55] The maximum possible number of clusters was set to six. The Calinski-Harabasz index (also called variance ratio criterion) is a measure of how dense the objects within a cluster are and how well different clusters are separated. The optimal number of clusters is the one which yields the highest value. The gap statistic compares the within cluster variation to its variation expected under the assumption of a reference null distribution.[55] A high gap value for a certain number of clusters indicates a large difference from the uniform distribution. These two measures indicated the optimal number of clusters based on the drawn trials. This was repeated 1000 times with newly drawn trials. After that, we calculated the frequency of how often the different cluster numbers were assigned among across the repetitions.

Population analyses

Population analyses were performed on the entire population of recorded neurons. All neurons with an average firing rate of at least 1 Hz and at least 30 correct trials for each sample line length entered the analysis without any pre-selection for category-selectivity (two-category task: $n = 348$, three-category task: $n = 278$). We used the firing rates within a 600 ms fixed window at the end of the delay (starting 400 ms after sample offset) to capture delay-activity which carries the category information needed to be available for the subsequent test phase and at the same time exclude late sample-related responses.

A correlation matrix was created to visualize the firing rate differences between pairs of stimuli and to detect coding patterns. The strength of the relationship between the firing rates to two stimuli was measured by the correlation coefficient r (deviation from the regression line). For that, the firing rates of each neuron were normalized by subtracting the average baseline firing rate (measured within 300 ms before sample onset across all correct trials) and dividing by the respective standard deviation. The coefficients of each correlation were represented as a tile in the correlation matrix. The tiles along the diagonal from lower left to upper right represent the correlation of the stimuli with themselves ($r = 1.0$). The matrix is symmetric to the diagonal.

For quantification, we calculated the mean of the correlation coefficients for the relationships of stimuli which belong to the same category and for stimuli of different categories. Regarding the data from the two-category task, correlations within the same category were: S1 vs. S2, S1 vs. S3, S2 vs. S3, L1 vs. L2, L2 vs. L3 and L1 vs. L3 and correlations between different categories: S1 vs. L1, S1 vs. L2, S1 vs. L3, S2 vs. L1, S2 vs. L2, S2 vs. L3, S3 vs. L1, S3 vs. L2 and S3 vs. L3. In the three-category task, correlations within the same category were: S1 vs. S2, M1 vs. M2 and L1 vs. L2 and between different categories: S1 vs. M1, S1 vs. M2, S1 vs. L1, S1 vs. L2, S2 vs. M1, S2 vs. M2, S2 vs. L1, S2 vs. L2, M1 vs. L1, M1 vs. L2, M2 vs. L1 and M2 vs. L2. The mean difference between these values was statistically verified by a two-sample t-test.

To test whether the activity of the neuronal population is behaviorally relevant, we calculated the correlation coefficients also in error trials (i.e. when the crow responded to the ''nonmatch'' stimulus or to neither of the two test stimuli). For that, we used all neurons of the analyzed population which had additionally at least 3 error trials for each sample line length (two-category task: $n = 172$, three-category task: $n = 120$). Baseline activity and respective standard deviation for firing rate normalization was measured in error trials within 300 ms before sample onset. Further analysis was done equally as for correct trials.

Additionally, we tested how well a multi-class support vector machine (SVM) classifier categorizes the firing rates of the recorded neurons. We used the LIBSVM toolbox for MATLAB (version 3.24)[57] with default parameters (multi-class classification, radial basis function as kernel). We performed a 5-fold cross-validation with the firing rates of the neuronal population to the six different sample line lengths within the last 600 ms of the delay. For that we randomly drew the firing rates of 30 trials of each neuron for each sample line length and assigned the true category labels to them. Then the drawn set of firing rates was normalized by z-scoring and then split into five equal groups. The classifier was trained with the firing rates of four fifth of the trials (144 trials of each neuron, sample stimuli were balanced, i.e. 24 trials for each sample stimulus) and then tested with the remaining one fifth (36 trials, i.e. 6 trials for each sample stimulus). This procedure was repeated five times so that each split of firing rates was used once as the test set, resulting in one accuracy value per sample line length (percentage of correctly classified firing rates across the five repetitions). Furthermore, we repeated the 5-fold cross-validation with 30 newly drawn trials for each sample line length 1000 times. The resultant confusion matrix shows the averaged classification probability across the trial samplings for the firing rates of each sample line length.

We finally used the SVM classifier to test whether the activity of the neurons to a single stimulus of each category of the two-category task can be used to predict the correct category of firing rates to the remaining stimuli which were not used for training. To that aim, we used three different sets of training and testing stimuli. First, we trained the classifier using the firing rates to the stimuli S1 and

L3 (most distant from the category boundary) and predicted the category of the stimuli S2, S3, L1 and L2. Next, we used the stimuli S2 and L2 for classifier training and S1, S3, L1 and L3 for the prediction and finally, S3 and L1 (adjacent to the category boundary) were used for training and S1, S2, L2 and L3 as prediction stimuli. For the classifier training and testing, we randomly drew the firing rates of 30 trials per line length and normalized these by z-scoring. Then we calculated the percentage of how often the classifier predicted that the test firing rates belong to the ''short'' category. This was repeated 1000 times with newly drawn firing rates. The results were then averaged across the repetitions.

Study 6: A neural correlate of sensory consciousness in a corvid bird

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CONSCIOUSNESS

A neural correlate of sensory consciousness in a corvid bird

Andreas Nieder*, Lysann Wagener, Paul Rinnert

Subjective experiences that can be consciously accessed and reported are associated with the cerebral cortex. Whether sensory consciousness can also arise from differently organized brains that lack a layered cerebral cortex, such as the bird brain, remains unknown. We show that single-neuron responses in the pallial endbrain of crows performing a visual detection task correlate with the birds' perception about stimulus presence or absence and argue that this is an empirical marker of avian consciousness. Neuronal activity follows a temporal two-stage process in which the first activity component mainly reflects physical stimulus intensity, whereas the later component predicts the crows' perceptual reports. These results suggest that the neural foundations that allow sensory consciousness arose either before the emergence of mammals or independently in at least the avian lineage and do not necessarily require a cerebral cortex.

Sensory consciousness, the ability to have subjective experience that can be explicitly accessed and thus reported, arises from brain processes that emerged through evolutionary history (*1*, *2*). Today, the neural correlates of consciousness are primarily associated with the workings of the primate cerebral cortex (*3*–*6*), a part of the telencephalic pallium that is laminar in organization (*7*–*9*). Birds, by contrast, have evolved a different pallium since they diverged from the mammalian lineage 320 million years ago (*10*, *11*). The bird pallium retains organizational principles reminiscent of the mammalian brain (*12*) but is distinctively nuclear and lacks a layered cerebral cortex (*13*–*15*). Despite this, birds demonstrate sophisticated perceptual and cognitive behaviors that suggest conscious experiences (*16*, *17*).

The associative endbrain area called nidopallium caudolaterale (NCL) is linked to high-level cognition in birds (*18*, *19*) and is considered a putative avian analog of the mammalian prefrontal cortex (*20*), which plays a predominant role in sensory consciousness in primates (*21*–*23*). To signify a "neural correlate of consciousness" in primates, brain activity that systematically changes with the subject's report of whether or not it had perceived identical stimuli is identified (*24*, *25*). We hypothesized that conscious experience originates from activity of the NCL in corvids and used a corresponding experimental protocol in which only the crows' internal state, not the physical stimulus properties, determined their subjective experience.

We trained two carrion crows (*Corvus corone*) to report the presence or absence of visual stimuli around perceptual threshold in a rule-based delayed detection task (Fig. 1A and supplementary materials and methods). At perceptual threshold, the internal state of the crows determined whether stimuli of identical intensity would be seen or not perceived. After a delay, a rule cue informed the crow about which motor action was required to report its percept. Thus, the crows could not prepare motor responses prior to the rule cues, which enabled the investigation of neuronal activity related to subjective sensory experience and its lasting accessibility.

The crows' proportion of "yes" responses in relation to increasing stimulus intensity gave rise to classical psychometric functions (Fig. 1,

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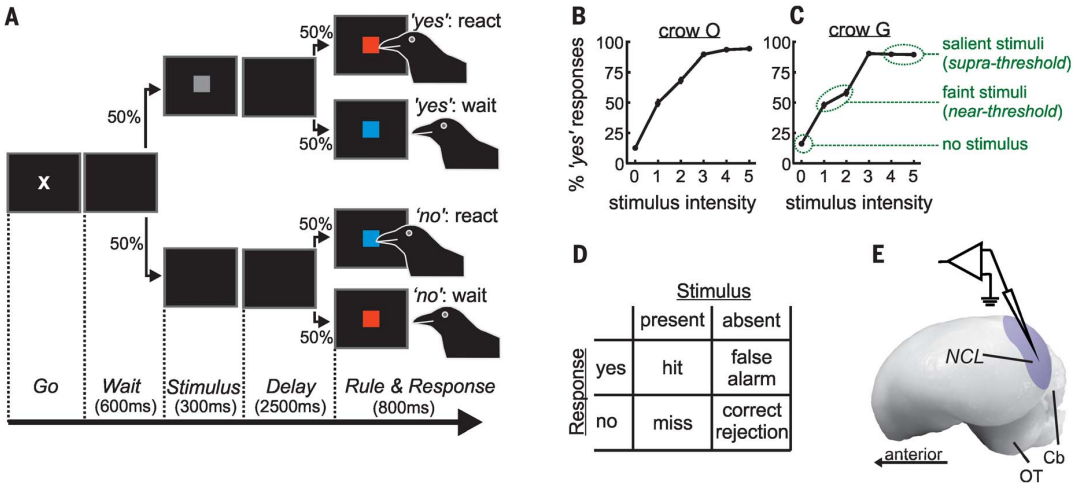

Fig. 1. Crows performed a delayed stimulus detection task. (**A**) Behavioral task. After the crow initiated a trial in the Go period, a brief visual stimulus of variable intensity appeared in 50% of the trials (stimulus trials), whereas no stimulus appeared in the other half of the trials (no stimulus trials). After a delay period, a rule cue informed the crow how to respond if it had seen or had not seen the stimulus. In stimulus trials (top), a red cue required a response for stimulus detection ("yes"), whereas a blue cue prohibited a response for stimulus detection. In no-stimulus trials (bottom), rule-response contingencies were inverted. (**B** and **C**) Psychometric functions of crow O (B) and crow G (C). Grouping of trials into suprathreshold, near-threshold, and no-stimulus trials. Error bars (very small) indicate standard error of the mean. (**D**) Signal detection theory classifies an observer's behavior at detection threshold, given two stimulus conditions (stimulus present or absent) and two possible responses ("yes, stimulus present" and "no, stimulus absent"). (**E**) Lateral view of a crow brain depicting the nidopallium caudolaterale (NCL, shaded) in the telencephalon. Cb, cerebellum; OT, optic tectum.

B and C). Trials were classified into suprathreshold (the two highest stimulus intensities), near-threshold (the two lowest stimulus intensities at perceptual threshold), and no-stimulus categories (Fig. 1C). The crows' responses were classified according to signal detection theory into "hit" (correct "yes" response to a stimulus), "correct rejection" (correct "no" response for stimulus absence), "miss" (erroneous "no" response to stimulus presence), and "false alarm" (erroneous "yes" response for stimulus absence) (Fig. 1D).

While the crows performed the task, we recorded single-cell activity of 480 neurons (n = 306 for crow G; n = 174 for crow O) from the NCL (Fig. 1E and supplementary materials and methods). We first identified 262 task-selective neurons that showed differences in firing rates for suprathreshold trials versus no-stimulus trials (Mann-Whitney U test, $p < 0.01$). The selective time intervals of these neurons that together bridged the total trial period were classified into stimulus-related (n = 155) (Fig. 2A) and delay-related (n = 165) (Fig. 2B).

Next, we compared the discharges during the crows' "yes" versus "no" responses in the different trial categories (Fig. 1C and supplementary materials and methods). If neurons signal stimulus intensity, the responses to near-threshold stimuli should be indistinguishable irrespective of the crow's response. In addition, the responses during "false alarms" are expected to be similar to "correct rejections" in the no-stimulus condition. However, if neurons represent the crows' percept, they are expected to change activity as a function of the crows' later report. In this case, firing rates to near-threshold "no" responses should resemble those during "correct rejections" in no-stimulus trials. Likewise, discharges for near-threshold "yes" responses and "false alarms" should be more similar to those of suprathreshold "yes" responses.

During stimulus presentation, neurons responded mainly to stimulus intensity and only mildly to the crow's later reported conscious percept. The example neuron in Fig. 2C discharged exclusively to the presentation of a salient stimulus, without a correlation with the crow's "yes/no" responses. The neuron in Fig. 2D showed some correlation with the crow's later report because firing rates during near-threshold "yes" responses were similar to supra-threshold "yes" responses, whereas discharges during near-threshold "no" responses resembled "correct rejections."

During the subsequent delay period, however, many neurons responded according to the crows' impending report, rather than to stimulus intensity. The neuron in Fig. 2E showed categorically higher firing rates for all "yes" responses (suprathreshold and near-threshold "hits," as well as "false alarms" in the absence of stimuli) compared to all "no" responses ("no"

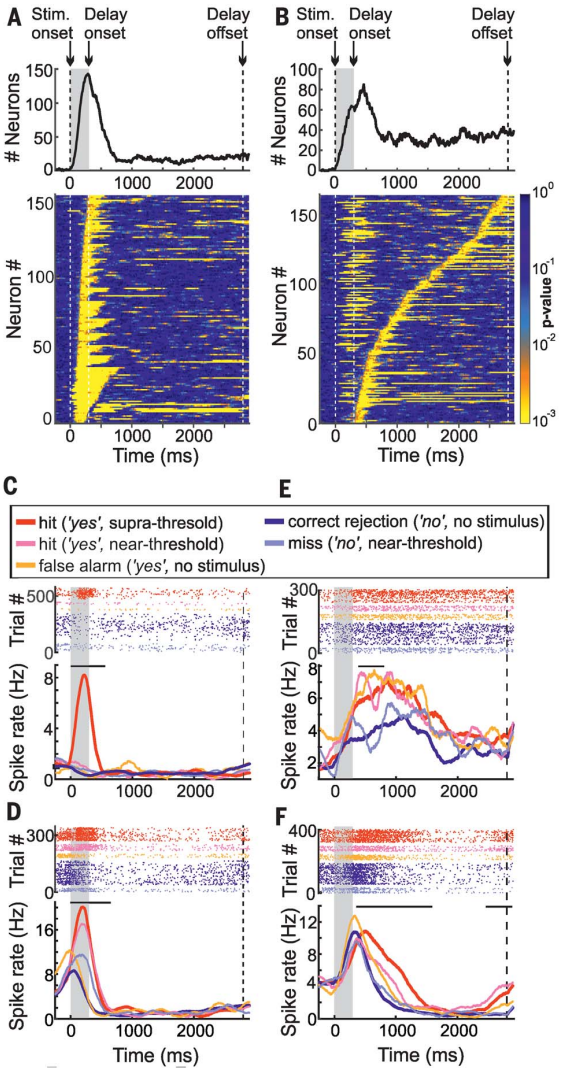

Fig. 2. Single-neuron responses in NCL. (**A** and **B**) Pattern of task selectivity for all stimulus-selective neurons during the stimulus (A) and delay period (B). Bottom: Color-coded traces of significance values (every line represents a neuron); neurons sorted according to selectivity latency. Top: Cumulative time-resolved histogram of task-selective intervals. (**C** and **D**) Activity of two stimulus-period task-selective example neurons in relation to the crow's behavioral responses. Top panels depict dot raster histograms (every line is a trial, every dot is an action potential); bottom panels represent the corresponding averaged and smoothed spike density histograms. The vertical gray shading indicates the presentation of the stimulus (onset at 0 ms), the vertical dotted line signifies the end of the delay. The color code represents the five different trial categories, with red, orange, and pink colors representing "yes" response trials, and dark and light blue colors "no" response trials. The horizontal bars in each spike-density histogram signify the task-selective interval. (**E** and **F**) Activity of two delay-period task-selective example neurons in relation to the crow's behavioral responses. Same layout as in (C) and (D).

responses to near-threshold stimuli, "correct rejections" in the absence of stimuli) during the first half of the delay period. A similar effect can be witnessed for the neuron in Fig. 2F, which showed discharges that correlated with the report at the beginning and end of the delay period.

To find out whether the activity of the 262 task-selective neurons was related to the crows' report for the same near-threshold stimuli, we compared the firing rates in the neurons' respective selectivity intervals for different trial outcomes. We used receiver operating characteristic (ROC) analysis from signal detection theory (*26*) (supplementary materials and methods). We derived the area under the ROC curve (AUC), termed choice probability, as the probability of predicting the subjective "yes/no" responses for identical stimuli for the stimulus and the delay phases separately (*27*).

We first compared the mean (rectified) activity during "hit" and "miss" trials for near-threshold stimuli in the stimulus presentation period. Choice probability was higher than the chance level of 0.5 (mean: 0.55; $p < 0.001$; one-sample Wilcoxon signed-rank test; n = 155 neurons; compared to a mean of 0.69 for suprathreshold "hits" and no-stimulus "correct rejections") (Fig. 3A). In addition, we compared the choice probability for "correct rejections" and "false alarms" during no-stimulus trials, which was comparable to chance (mean: 0.51; p = 0.08; one-sample Wilcoxon signed-rank test; n = 155 neurons) (Fig. 3B). Thus, during

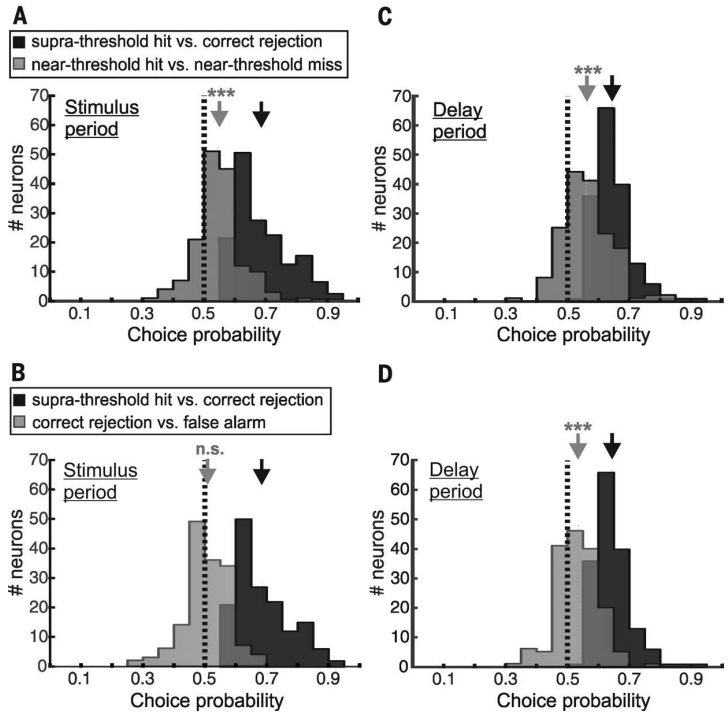

Fig. 3. Neuronal activity predicts "yes" versus "no" responses. Distribution of neuronal choice probabilities according to signal detection theory. (**A** and **B**) Choice probabilities during the stimulus period (155 neurons). (**C** and **D**) Choice probabilities during the delay period (165 neurons). Gray arrow indicates mean of choice probabilities for near-threshold hits versus near-threshold misses [(A) and (C)] and for correct rejections versus false alarms, respectively [(B) and (D)]. Choice probabilities in (A), (C), and (D) were significantly larger than chance level indicated by dotted vertical line (***$p < 0.001$; n.s., not significant). Black arrows indicate mean AUC values for suprathreshold hits versus correct rejections for comparison.

stimulus presentation, the neurons signaled the crows' subsequent report only mildly.

However, the primarily stimulus-based activity changed to a predominantly report-driven representation during the delay. Both the choice probabilities for near-threshold "hit" and "miss" trials (mean: 0.56; Fig. 3C), as well as the choice probability for no-stimulus "correct rejections" and "false alarms" (mean: 0.53; Fig. 3D), were higher than expected by chance ($p < 0.001$ for both values; one-sample Wilcoxon signed-rank test; $n = 165$ neurons). On the background of a mean AUC of 0.64 for suprathreshold "hits" and no-stimulus "correct rejections," both choice probabilities predicted the crows' perceptual report rather than the physical stimulus. Notably, this effect was found not only for the very same faint stimuli, but also on "false alarm" trials, when the crows mistakenly reported perceiving a stimulus when in fact no stimulus was present. Thus, shortly after stimulus presentation, the neurons represented the crows' later report.

To explore the time course of choice prediction from stimulus onset to delay offset irrespective of neuronal selectivity, we applied time-resolved population analyses based on the activity of all NCL neurons with sufficient trials per trial type ($n = 152$). We first trained a support vector machine (SVM) classifier to discriminate "yes" versus "no" responses on the basis of the spiking activity (*28*) (supplementary materials and methods). Cross-validation on "hits" in suprathreshold trials and "correct rejections" in no-stimulus trials indicated reliable information differentiating the crows' alternative responses (fig. S1). To minimize the influence of stimulus intensity, we next trained the classifier with discharges exclusively from near-threshold trials in which crows subjectively made "yes" and "no" responses for identical stimulus intensities. After training, the classifier was tested with new data from the same neuronal population, but for suprathreshold "hits" versus "correct rejections" in the absence of stimuli. Indeed, the classifier was able to correctly assign the new trials into "yes" versus "no" responses, with particularly high accuracy at stimulus offset and toward the end of the delay (Fig. 4A). This indicates that a population of neurons contained information about the crows' subjective experience throughout the trial.

Finally, we quantified how much information about the physical stimulus and the later report was carried by the activity of the same population of NCL neurons across the trial. We calculated the percent explained variance (ω^2, PEV) for stimulus intensity and "yes/no" response (*29*, *30*) (supplementary materials and methods). We found that stimulus intensity information increased sharply after stimulus presentation, but then rapidly decayed and vanished during the following delay (Fig. 4B). Instead, the neurons increasingly encoded the crows' perceptual report until it reached a peak level toward the end of the delay (Fig. 4B). A similar response pattern was found for predictions on near-threshold trials of a SVM-classifier trained on population responses of "yes" responses in suprathreshold trials ("hits") and "no" responses in no-stimulus trials ("correct rejections") (fig. S2). The neuronal population results suggest that NCL neurons switch from initially mainly representing stimulus intensity to predominantly encoding the crows' subjective experience later in the trial and before a required behavioral report.

A difference between the neuronal activities of one reported perceptual state versus the other for equal visual stimuli is considered to be a "neural correlate of visual consciousness" (*3*, *5*, *21*–*23*). Our finding thus constitutes an empirical marker of avian sensory consciousness. As for any animal, the qualitative nature of this subjective experience—"what it is like" for a crow to be consciously aware of sensory data—remains hidden (*31*). Moreover, whether pure subjective experience itself ("phenomenal consciousness") can and should be dissociated from its report ("access consciousness") remains intensely debated (*1*, *32*).

Our report of a two-stage process in awareness in the corvid NCL is markedly similar to findings in the primate cerebral cortex, where the initial sweep of activity is also mainly involved in unconscious vision, whereas activity correlating with consciousness is delayed relative to stimulus onset activity (*21*, *33*–*36*). To explain these effects, the global neuronal workspace theory (*25*, *37*) posits that only sensory activity that is strong enough can access awareness by causing a state termed "global ignition" in higher brain centers such as prefrontal cortex. "Ignition" causes information about a brief stimulus to become sustained and broadcasted back through recurrent interactions between many brain areas, thereby also characterizing the transition of a sensory representation into the explicit working memory state (*1*, *23*). The NCL may very well constitute the avian brain site of an "all-or-none" ignition process that leads either to a high degree of activation causing and maintaining

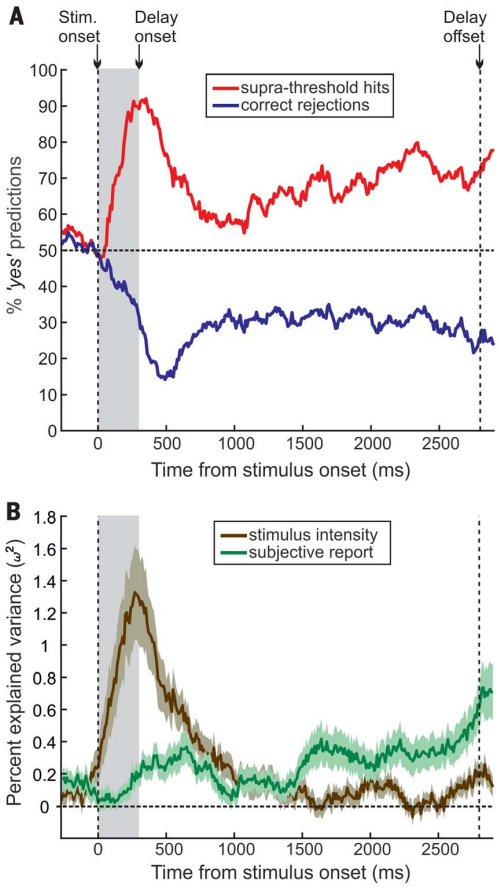

Fig. 4. Time-resolved neuron population analyses. (**A**) A support vector machine (SVM) classifier trained on near-threshold trial activity predicts the crows' "yes" responses from suprathreshold "hit" trials and "no" responses from correct rejection no-stimulus trials. Chance level is 50%. (**B**) Sliding-window percent explained variance (ω^2) analysis quantifying the information about the stimulus intensity and report-associated subjective percept.

information about conscious experience across a temporal gap for a future goal, or to a vanishing response. Combining report-based behavioral protocols in crows with no-report protocols may help to disentangle the neural mechanisms involved in generating, maintaining, and reporting conscious experience (*38*, *39*). This two-stage process in awareness could prove to be a general and evolutionarily stable principle of how sensory consciousness is achieved in vertebrates in general.

Our finding also provides evidence for the phylogenetic origins of consciousness (*2*). It excludes the proposition that only primates or other mammals possessing a layered cerebral cortex are endowed with sensory consciousness. To reconcile sensory consciousness in birds and mammals, one scenario would postulate that birds and mammals inherited the trait of consciousness from their last-common ancestor. If true, this would date the evolution of consciousness back to at least 320 million years when reptiles and birds on the one hand, and mammals on the other hand, evolved from the last common stem-amniotic ancestor (*40*). Alternatively, consciousness emerged independently on the basis of convergent evolution on different branches of the vertebrate "tree of life." According to this hypothesis, consciousness was absent in the common stem-amniotic ancestor, but—comparable to homeothermy—evolved later and independently during the rise of, at least, birds and mammals. Yet another scenario would predict a gradual emergence of consciousness. Here, different degrees of conserved pallial connectivity patterns in vertebrates could give rise to aspects of sensory consciousness across phylogeny. Combining measurements of brain signals with controlled behavioral protocols will help to delineate the origins of conscious experience in the animal kingdom.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/369/6511/1626/suppl/DC1
Materials and Methods
Figs. S1 and S2

View/request a protocol for this paper from *Bio-protocol*.

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Supplementary Materials for

A neural correlate of sensory consciousness in a corvid bird

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This PDF file includes:

Other Supplementary Materials for this manuscript include the following:
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MDAR Reproducibility Checklist (.pdf)

Materials and Methods

Subjects

We used two 1-year-old male carrion crows (*Corvus corone*; bird G and bird O) from the institute's breeding facility. They were hand-raised and housed in social groups of four animals in indoor aviaries. The crows were on a controlled feeding protocol during the training and recording periods, with body weight measured daily. The daily amount of food was given as reward during, or if necessary, after the sessions. Water was provided *ad libitum* in the aviaries and during the experiments. All procedures were carried out according to the guidelines for animal experimentation and approved by the responsible national authorities, the Regierungspräsidium Tübingen, Germany.

Experimental setup

The experiment was conducted in a darkened operant conditioning chamber. The birds were perched in front of a touchscreen monitor (ART development MT1599-BS) that was used for stimulus presentation. Rewards were delivered by an automated feeder below the touchscreen. The food reward consisted of food pellets (*Beo Special*, Vitakraft, Bremen or *NutriBird Beo komplet*, Versele Laga, Belgium) and mealworms (*Tenebrio molitor* larvae). Additional visual feedback was provided by a lamp on top of the feeder and auditory feedback by speakers (*Lasmex S-03*) located behind the touchscreen. An infrared light barrier controlled by a reflector attached to the bird's head ensured a stable head position in front of the screen throughout the trial. We used the CORTEX system (National Institute of Mental Health) to carry out the experiment and collect behavioral data. Neuronal data was recorded using a PLEXON system (Plexon Inc., Dallas, Texas).

Behavioral protocol

The crows were trained to report the presence or absence of a visual stimulus (square) in the center of a black computer screen (**Fig. 1A**). Only correct responses were rewarded. It was imperative to work with highly-trained crows because the crows needed to continue to respond in agreement with the task contingencies even when the stimuli where difficult to detect at near-threshold. Due to the task difficulty in near-threshold trials, the crows needed to pay close attention to all trials.

The crow initiated a trial by positioning its head facing the monitor whenever a go-stimulus (small white cross, 2x2° visual angle) was shown, thus closing an infrared light barrier, and maintaining this position throughout the trial. To indicate that the light barrier had been entered, the bird heard a click sound, the cross turned briefly into a circle (60ms), and the go-stimulus vanished. Whenever a crow made premature head movements and thereby left the light barrier during an ongoing trial, this trial was terminated and discarded.

The main protocol started with a black screen for 600ms (wait period) after which the stimulus period followed. In the stimulus period, a grey square (4.5° of visual angle) was shown in 50% of the trials, whereas no stimulus was presented in the remaining 50%. The stimulus was presented at six levels of intensity close to the perceptual threshold. The intensity of the stimuli was individually adjusted so that the two faintest stimulus values were at threshold (around 50% 'yes' responses), whereas the two highest values were salient and always detectable. Whether a stimulus was shown or not, and which intensity the stimulus

had, was shuffled pseudo-randomly on a trial by trial basis by the computer running the task. The stimulus period was followed by a 2,500ms delay period with a blank screen, after which a rule cue (colored square) was shown.

The implementation of a response rule at the end of the trial prevented the crows from preparing a response and thus avoided confounding neural activity correlated with sensory consciousness with preparatory motor neuronal activity throughout the delay period. The rule cue informed the crow how to respond as a function of whether it had or had not seen a stimulus. If a stimulus was present, a red rule-cue required the crow to respond (i.e. to move the head out of the light barrier within 800ms) to earn a reward, whereas a blue rule-cue demanded the crow withhold from responding and maintain stable head position in the light barrier for 800ms to receive a reward. The orthogonal rule-response relationships were applied for the absence of a stimulus. If a stimulus was absent, a red rule-cue required the crow to withhold from responding, whereas a blue rule-cue demanded the crow quickly respond. To know whether to respond or withhold from responding, the crow needed to combine its conscious experience about the stimulus with the conditional instruction signified by the rule cues. Because the response rule cues were pseudo-randomized, fully balanced and unbeknownst to the crow at the beginning of each trial, the crow could not benefit from preparing a motor response: it would have been correct in only 50% of the trials. This chance implementation of required responses prevented the crow from learning stimulus-response associations or any attempt to plan its response during the delay.

Surgery and Recordings

The surgery was performed while the animal was under general anesthesia with a mixture of ketamine (50mg/kg) and Rompun (5mg/kg xylazine). The animal was placed in a stereotaxic holder. We targeted the medial part of NCL (*Nidopallium caudolaterale*) by performing a craniotomy at 5mm anterior-posterior and 13mm medio-lateral on the left hemisphere of both birds. Two manual micro drives containing four electrodes each (2MΩ, Alpha Omega Co.) were implanted at the craniotomy. In addition, a miniature connector for the headstage and a small holder for attaching the reflector were implanted. Each recording session started with adjusting the electrodes until a proper neuronal signal was detected on at least one channel. The neurons were never pre-selected for any involvement in the task. Single-cell separation was done offline (Plexon Offline Sorter, version 2.6.2).

Behavioral analysis

For the behavioral analysis we gathered 'yes' and ‘no’ responses for every single trial (18,548 trials for crow G; 22,447 trials for crow O) during the recording sessions (41 for crow G and 37 for crow O). For both response types, trials requiring head movements or no head movements according to the response rules were pooled. The proportion of ‘yes’ responses was plotted as a function of stimulus intensity to give rise to a sigmoidal psychometric function.

Neuronal analysis

Data analysis was performed using MATLAB (MathWorks, Natick, MA, USA). For neuronal analyses, the trials were classified into three different trial categories according to the crows’ psychophysical performance correlating with stimulus intensity.

No-stimulus trials: Stimulus intensity 0 corresponds to the 50% of trials in which no stimulus was presented. 'No' responses correspond to 'correct rejections', whereas 'yes' responses signify 'false alarms'.

Supra-threshold trials: Salient stimuli presented at intensities 4 and 5 were supra-threshold conditions in which the crows detected the stimulus in almost 100% of the trials. The crows produced almost exclusively 'yes' responses (i.e. 'hits') for such supra-threshold stimuli.

Near-threshold trials: Stimulus intensities 1 and 2 represent faint intensities near the perceptual detection threshold (~50% 'yes' responses) of the crows. In such trials, the subjective perception of the crows resulted either in 'yes' ('hit') or 'no' responses ('miss').

All neurons that had an average firing rate of at least 0.5 Hz, and were recorded for at least 4 trials of the above mentioned trial categories and responses ('hits' in supra-threshold trials, 'hits' and 'misses' in near-threshold trials, and 'correct rejections' and 'false alarms' in no-stimulus trials) entered analysis.

We first identified task-selective cells and their selective time intervals. We compared individual neurons' activity to the most unambiguous stimulus conditions, namely 'correct rejections' in the no-stimulus trials with 'hits' in the supra-threshold trials. For that, we used a sliding Mann-Whitney U test (200ms window duration, 10ms step size, $p<0.01$) beginning at sample onset and ending 100ms after delay offset. If neuronal activity significantly differed over at least 11 consecutive windows (i.e. 300ms in total), this interval and the neuron was termed task selective. Task-selective intervals occurring between stimulus onset until 300ms after stimulus offset were classified as stimulus-related; all later occurring selective intervals were classified as delay-related. Activity in the stimulus and delay period were subsequently separately analyzed. If a neuron had more than one selective time interval in the stimulus and delay period, respectively, only the one with the greater difference in firing rate to supra-threshold 'hit' trials versus 'correct rejections' in no-stimulus trials (interval with the smallest p-value of the Mann-Whitney U test) was used.

Average and time-resolved firing across a trial per single neuron is depicted by dot raster histograms (every line corresponds to a trial; every dot represents an action potential) and spike density functions. Spike density functions were averaged over trials and convolved with a Gaussian kernel (bin width 300 ms, step size 1 ms) for illustrative purposes only.

Receiver operating characteristic (ROC) analysis.

To investigate if neurons represent the crows' conscious percept, we applied receiver operating characteristics (ROC) analysis separately for the stimulus and delay periods (*26*). For that, we used individual neurons' firing rates from the previously identified task selective intervals.

We compared two conditions that are informative about whether or not neuronal activity varies with consciousness. First, we compared firing rates during 'hits' and 'misses' for near-threshold trials. Second, we compared 'false alarms' with 'correct rejections' in no-stimulus trials. If neurons signal the crows' percept, they are expected to discriminate between those trial categories, and with qualitatively similar firing rates as for supra-threshold 'hits' versus no-stimulus 'correct rejection' trails.

To estimate the extent to which neuronal activity in both phases was influenced by the subjective experience, we calculated the area under the receiver-operating-characteristics curve (ROC), termed area under the ROC curve (AUC), derived from signal detection theory. The AUC is a quantitative measure of how well a neuron based on its firing rates

discriminates between two conditions. A value of 0.5 indicates chance level, whereas a value of 1.0 denotes perfect discriminability. As a reference, we calculated the AUC for task-selective neurons between supra-threshold 'hits' ('yes' response), and 'correct rejections' in the absence of a stimulus ('no' response). If a task-selective neuron showed suppression during 'yes' responses (which leads to AUC values <0.5), the AUC was rectified (i.e. mirrored at 0.5) so that both negative and positive deflections resulted in values greater than 0.5. Accordingly, the other AUC values of such a neuron were also mirrored at 0.5.

The AUC calculated for identical stimulus situations leading to different behavioral outcomes is termed choice probability index (*27*). Choice probability indicates how well a neuron predicts the crow's subjective 'yes/no' responses for identical stimulus intensities. Choice probabilities were calculated for 'hits' versus 'misses' for near-threshold stimuli, as well as 'correct rejection' and 'false alarms' in the no-stimulus condition. If the reference AUC of a task-selective neurons between supra-threshold 'hits' and 'correct rejections' required rectification to yield values >0.5, the respective choice probabilities were also rectified, irrespective of their values. The reference AUC and the two choice probabilities were calculated for the stimulus and the delay phases separately.

Percent explained variance analysis

We quantified the time course of information about stimulus intensity and subjective 'yes' versus 'no' responses carried by the firing of the entire population of neurons, irrespective of task selectivity. To that aim, we used a sliding-window percent explained variance analysis (ω^2, PEV) (*29,30*). Only neurons for which at least 30 trials for each of the above mentioned trial categories ('hits' in supra-threshold trials, 'hits' and 'misses' in near-threshold trials, and 'correct rejections' and 'false alarms' in no-stimulus trials) could be recorded were used (152/480 neurons). We used a 400ms sliding window (10-ms step size), and a two-factorial ANOVA was calculated for each window. The resultant sums of squares for each ANOVA were used to estimate the percentage of variance attributable to either the stimulus intensity or the 'yes'/'no' response for each neuron as a function of time. The ω^2 was calculated using

$$\omega^2 = \frac{SS_{factor} - df * MSE}{SS_{total} + MSE}$$

where SS_{factor} was the sum of squares for the factor stimulus intensity and subjective percept ('yes'/'no' response), respectively, SS_{total} was the total sum of squares, df the degrees of freedom and MSE the mean squared error. All neurons were then averaged together, yielding a population estimate of the average percentage of variance explained by each factor.

***Support vector machine* (SVM) classifier**

To investigate whether the decision in no-stimulus trials and supra-threshold trials can be predicted by the activity of the neuronal population in near-threshold trials, we used a support vector machine classifier (*28*). Only neurons for which at least 30 trials for each of the above mentioned trial categories ('hits' in supra-threshold trials, 'hits' and 'misses' in near-threshold trials, and 'correct rejections' and 'false alarms' in no-stimulus trials) could be recorded entered this analysis (152/480 neurons). We trained the classifier in sliding windows (400 ms window, steps 10 ms) on the firing rates of 'yes' and 'no' responses in near-threshold trials to exclude the factor stimulus. For each sliding window we used the firing rates of all

neurons in 30 randomly drawn near-threshold ‘hit’ trials and near-threshold ‘miss’ trials, respectively.

The trained classifier was then used to predict the label of 30 randomly drawn supra-threshold ‘hit’ trials and 30 no-stimulus ‘correct rejection’ trials. We calculated the percentage of ‘yes’-predictions as a measure for decision information in the predicted trials. We repeated the classifier training and prediction 1000 times with 30 newly drawn trials for each trial category to account for variability in trial selection and calculated the mean proportion of ‘yes’-predictions.

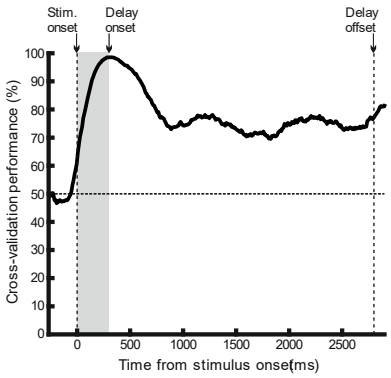

Fig. S1. SVM classifier cross-validation. To verify that the SVM classification accuracy remains stable across trials, we performed a sliding window 5-fold cross-validation (400 ms window with steps of 10 ms) on 'yes' responses in supra-threshold trials ('hits') and 'no' responses in no stimulus trials ('correct rejections'). The trials (30 'hit' and 30 'correct rejection' trials) were split into five equally sized groups of which four were used to train a SVM classifier to predict the label of the remaining group. This process was repeated until all five groups were used once for prediction. Cross-validation therefore gives an estimate about the information inherent in the data. The cross-validation performance (proportion of correctly labeled trials) was then averaged across 1000 repetitions. The cross-validation performance increased steeply after stimulus onset peaking close to 100% during the time of stimulus presentation. During the delay period, the classifier was able to predict the correct trial labels with a performance of close to 80%. This indicates reliable neuronal information differentiating 'yes' responses in supra-threshold and 'no' responses in no stimulus trials throughout the trial.

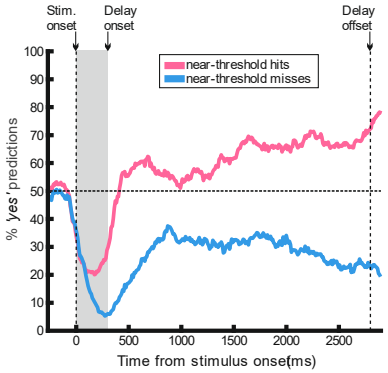

Fig. S2. Classifier prediction of near-threshold trials based on population responses to supra-threshold and no-stimulus conditions. An SVM classifier was trained on the firing rates of 'yes' responses in supra-threshold trials ('hits') and 'no' responses in no stimulus trials ('correct rejections'). This classifier was then used to predict the crows' choices in near-threshold trials. We used the same population of 152 neurons and the same parameters (400 ms window with steps of 10 ms) as for the other classifier that was trained on the firing rates in near-threshold trials (**Fig. 4A**). The SVM classified the near-threshold trials during the stimulus presentation period as stimulus-absent trials. This was most likely due to the lack of strong sensory responses that were present during classifier-training in the supra-threshold conditions, but absent during classifier-testing with near-threshold stimuli. However, shortly after the stimulus presentation period, the classifier correctly predicted the crows' 'stimulus present'- versus 'stimulus absent'-choices. This indicates that NCL neurons switched from primarily representing stimulus-intensity at the very beginning of the trial to encoding the crows' conscious percept later in the trial.

Study 7: Conscious experience of stimulus presence and absence is actively encoded by neurons in the crow brain

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Conscious Experience of Stimulus Presence and Absence Is Actively Encoded by Neurons in the Crow Brain

Lysann Wagener and Andreas Nieder

Abstract

■ The emergence of consciousness from brain activity constitutes one of the great riddles in biology. It is commonly assumed that only the conscious perception of the presence of a stimulus elicits neuronal activation to signify a "neural correlate of consciousness," whereas the subjective experience of the absence of a stimulus is associated with a neuronal resting state. Here, we demonstrate that the two subjective states "stimulus present" and "stimulus absent" are represented by two specialized neuron populations in crows, corvid birds. We recorded single-neuron activity from the nidopallium caudolaterale of crows trained to report the presence or absence of images presented near the visual threshold. Because of the task design, neuronal activity tracking the conscious "present" versus "absent" percept was dissociated from that involved in planning a motor response. Distinct neuron populations signaled the subjective percepts of "present" and "absent" by increases in activation. The response selectivity of these two neuron populations was similar in strength and time course. This suggests a balanced code for subjective "presence" versus "absence" experiences, which might be beneficial when both conscious states need to be maintained active in the service of goal-directed behavior. ■

INTRODUCTION

How perceptual consciousness, the subjective experience associated with a reportable sensory event, emerges from the workings of the brain is a fundamental question in biology (Ehret & Romand, 2022; Vallortigara, 2021; Laureys, 2005; Nagel, 1974). The main method to study how neurons give rise to perceptual consciousness relies on identifying neuronal activity that specifically occurs during subjective reports of the subject under study. Such "neural correlates of consciousness (NCCs)," defined as the minimal set of neuronal events and mechanisms sufficient for a specific conscious percept (Koch, Massimini, Boly, & Tononi, 2016), have been explored in humans (Pereira et al., 2021; Gelbard-Sagiv, Mudrik, Hill, Koch, & Fried, 2018; Reber et al., 2017; Quiroga, Mukamel, Isham, Malach, & Fried, 2008; Kreiman, Fried, & Koch, 2002), in nonhuman primates (Kapoor et al., 2022; van Vugt et al., 2018; Panagiotaropoulos, Deco, Kapoor, & Logothetis, 2012; de Lafuente & Romo, 2005; Leopold & Logothetis, 1996; Logothetis & Schall, 1989), and recently also in the crow, a corvid songbird (Nieder, Wagener, & Rinnert, 2020). Common to all these experimental approaches is that physically identical stimuli spontaneously elicit one of two contrasting, endogenously generated percepts. The general finding is that a proportion of neurons in higher-order brain areas becomes active in relation to the subject's alternating conscious percept for physically identical stimuli. This holds even when reports are initially undefined to the subject or not required, arguing that the activity of such neurons represents the subjective experience and not factors related to the impending report (Kapoor et al., 2022; Hesse & Tsao, 2020; Nieder et al., 2020).

One of the most radical contrasts in subjective experience can be witnessed when stimuli are presented near perceptual threshold of the subject (Nieder et al., 2020; van Vugt et al., 2018; de Lafuente & Romo, 2005). Despite the constant intensity of the target stimulus across trials, the perceptually ambiguous stimulus is sometimes perceived, whereas other times, the stimulus is not perceived. In other words, conscious perception switches between conscious "stimulus-present" and "stimulus-absent" states, irrespective of the constant intensity of the stimulus. As an NCC, neurons respond in relation to the changing perceptual states. Thus, the readout of neuronal activity can predict whether the subject was consciously aware or unaware of the stimulus (Nieder et al., 2020; van Vugt et al., 2018; Quiroga et al., 2008; de Lafuente & Romo, 2005).

The tacit assumption from these studies is that the NCC is only based on percept-related neurons that show elevated firing rates for perceived stimuli but remains silent when stimulus absence is experienced (van Vugt et al., 2018; Quiroga et al., 2008; de Lafuente & Romo, 2005). Within this framework, only conscious perception is encoded, whereas the absence of a percept correlates with neurons' resting activity (Pereira, Perrin, & Faivre, 2022). However, it is conceivable that not only subjective experience of the presence of a stimulus is encoded by neuronal

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activity but also the experience about the absence of a stimulus (Pereira et al., 2022). After all, both stimulus presence and stimulus absence experiences constitute explicit categorical states.

Evidence supporting the intriguing possibility that also the conscious experience of stimulus absence can be encoded actively comes from single-neuron recordings in the associative cerebral cortex of monkeys and humans (Pereira et al., 2021; Merten & Nieder, 2012). Given that evidence for neurons actively signaling the experienced absence of stimuli stem exclusively from primates, one hypothesis is that this way of implementing conscious percepts might have emerged with the advent of a mammal-specific and computationally powerful layered neocortex. Alternatively, this way of representing two subjective states by two specialized neuron populations may constitute a computational advantage that therefore might be implemented in other vertebrate classes, such as birds, with distinctly evolved endbrains (telencephala) lacking a cerebral cortex (Jarvis et al., 2005) and neuronal circuits of distinct developmental origin (Colquitt, Merullo, Konopka, Roberts, & Brainard, 2021). Recently, we reported a neuronal correlate of perceptual consciousness in the associative endbrain area *nidopallium caudolaterale* (NCL) of carrion crows (Nieder et al., 2020). In the current study, we reanalyzed this data set to explore the hypothesis that—similar to the primate neocortex—the two subjective states "stimulus present" and "stimulus absent" are represented by two specialized neuron populations in the independently evolved telencephalon of birds (Nieder, 2021).

METHODS

Subjects

Two 1-year-old hand-raised male carrion crows (*Corvus corone*) from the institute's breeding facility were used. They were housed in a social group of four crows in an indoor aviary. During the experiment, the crows were on a controlled feeding protocol and received their daily amount of food as reward during training and recording or, if necessary, after the sessions. The body weight was measured daily. Water was available ad libitum during the experiments and in the aviary. All procedures were carried out according to the guidelines for animal experimentation and approved by the responsible national authorities, the Regierungspräsidium Tübingen, Germany.

Behavioral Protocol

We trained the crows to report the presence or absence of a gray visual stimulus (4.5° of visual angle) presented at six different intensity levels in the center of a black computer screen.

The experiment was conducted in a darkened operant conditioning chamber. The crow was placed on a perch in front of a touchscreen monitor (ART Development MT1599-BS), which was used only for stimulus presentation. The behavior and response of the crow were controlled by an infrared light barrier, which was located above the crow and registered the position of a reflector foil attached to the bird's head. Reward (either birdseed pellets or mealworms [*Tenebrio molitor* larvae]) was given by an automated feeder below the touchscreen. Auditory feedback was provided by speakers (Lasmex S-03) located behind the touchscreen. We used the CORTEX system (National Institute of Mental Health) to run the experiment and collect behavioral data.

The crow initiated a trial by positioning its head facing the screen whenever the go stimulus (small white cross, $2 \times 2°$ of visual angle) was shown. Auditory feedback indicated that the light barrier had been entered and the go stimulus turned briefly into a circle (60 msec) before it vanished. This stable head position had to be maintained throughout the trial until the response phase. Premature head movements aborted the ongoing trial, which was then discarded.

After a 600-msec waiting period in which the screen was completely black, the stimulus period followed. In 50% of the trials, the visual stimulus was shown for 300 msec, whereas in the other 50%, the screen remained black. The intensity of the stimulus was close to the perceptual threshold and individually adjusted so that the two faintest stimulus values were at threshold (around 50% "yes" responses) and the two highest values were salient and always detectable. Whether a stimulus was shown or not, and the intensity of the stimulus, was shuffled pseudorandomly on a trial-by-trial basis.

Then, the screen was black for a delay of 2500 msec, after which a rule cue (colored square) informed the crow how to respond. For a correct response, the crow needed to associate its conscious experience about the stimulus with the conditional instruction signified by the rule cue. If a stimulus was present, a red square required the crow to respond (i.e., to nod and thus move the head out of the light barrier within 800 msec) to earn a reward, whereas a blue square demanded withholding from responding and maintaining a stable head position for 800 msec to receive a reward. The orthogonal rule–response relationships were applied for the absence of a stimulus. If a stimulus was absent, a red square required the crow to withhold from responding, whereas a blue square demanded a response. The rule cues were pseudorandomized, fully balanced and unbeknownst to the crow at the beginning of each trial. This prevented the bird from learning stimulus–response associations and from preparing a motor response already during the stimulus and delay periods.

Surgery and Neurophysiological Recordings

The surgery was performed while the animal was under general anesthesia with a mixture of ketamine (50 mg/kg)

and xylazine (5 mg/kg). The animal was placed in a stereotaxic holder. We targeted the medial part of the NCL by performing a craniotomy at 5 mm anterior–posterior and 13 mm mediolateral on the left hemisphere of both birds (Kersten, Friedrich-Müller, & Nieder, 2022). Two manual micro drives containing four electrodes each (2 MΩ, Alpha Omega Co.) were implanted at the craniotomy. In addition, a miniature connector for the headstage and a small holder for attaching the reflector were implanted. After the surgery, the crows received analgesics. Each recording session started with adjusting the electrodes until a proper neuronal signal was detected on at least one channel. The neurons were never preselected for any involvement in the task. Neuronal data were recorded using the Plexon system (Plexon Inc.). Single-cell separation was done offline (Plexon Offline Sorter, Version 2.6.2).

Data Analysis

Behavior

Data analysis was performed using MATLAB (The MathWorks). We recorded behavioral and neuronal data during 37 sessions for Crow 1 and 41 sessions for Crow 2. During these sessions, the birds performed 22,447 (Crow 1) and 18,548 (Crow 2) single trials, respectively. The proportion of "yes" responses was plotted as a function of stimulus intensity to give rise to a sigmoidal psychometric function. For that purpose, trials with both response types (requiring head movements or no head movements according to the rule cue) were pooled.

Neuronal Analysis

We analyzed the data set that constituted the basis of a previous publication (Nieder et al., 2020). For neuronal analyses, the trials were grouped into three different trial categories according to the crows' psychophysical performance correlating with stimulus intensity:

Suprathreshold trials. Salient stimuli presented at Intensities 4 and 5 were suprathreshold conditions in which the crows detected the stimulus in almost 100% of the trials. The crows produced almost exclusively "yes" responses (i.e., "hits") for such suprathreshold stimuli.

Near-threshold trials. Stimulus Intensities 1 and 2 represent faint intensities near the perceptual detection threshold (~50% "yes" responses) of the crows. In such trials, the subjective perception of the crows resulted in either "yes" ("hits") or "no" ("misses") responses.

No-stimulus trials. Stimulus Intensity 0 corresponds to the 50% of the trials in which no stimulus was presented. "No" responses correspond to "correct rejections," and "yes" responses signify "false alarms."

All neurons that were used for the following analyses had an average firing rate of at least 0.5 Hz and were recorded for at least four trials of each trial category and responses mentioned above ("hits" in suprathreshold trials, "hits" and "misses" in near-threshold trials, and "correct rejections" and "false alarms" in no-stimulus trials). In addition, all neurons were task selective; that is, they had a time interval with a significant difference in their activity to the most unambiguous conditions, namely, "correct rejections" in the no-stimulus trials and "hits" in the suprathreshold trials. To identify this task-selective time window, we used a sliding Mann–Whitney U test (200-msec window duration, 10-msec step size, $p < .01$) beginning at sample onset and ending 100 msec after delay offset. A neuron was termed "task selective" if its neuronal activity differed over at least 11 consecutive windows (i.e., 300 msec in total). Task-selective intervals occurring between stimulus onset until 300 msec after stimulus onset were classified as stimulus related; all later occurring selective intervals were classified as delay related. If a neuron had more than one selective time interval during the sample and delay period, respectively, only the one with the greater difference in firing rate to suprathreshold "hit" trials versus no-stimulus "correct rejections" trials (interval with the smallest p value of the Mann–Whitney U test) was used.

We identified percept-related neurons, that is, task-selective neurons that showed a difference in firing rates to the crows' "yes'" versus "no" responses in near-threshold trials during their selective time windows, using receiver operating characteristics (ROCs). For that purpose, we calculated the area under the ROC curve (AUROC) as a measure of how well a neuron based on its firing rates discriminates between two conditions. A value of 0.5 indicates chance level, whereas a value of 1.0 denotes perfect discriminability. A percept-related neuron had to meet two criteria in unison: First, firing rates in suprathreshold "hit" compared to no-stimulus "correct rejection" trials had to be significantly different (i.e., task selectivity; Mann–Whitney U test, $p < .01$; see paragraph above). Second, AUROC values comparing near-threshold "hits" versus near-threshold "misses" had to be significantly different (permutation test, 1000 shuffled distributions, $p < .05$). If neuronal activity was smaller in "hits" compared to "correct rejections," the AUROC values were smaller than 0.5. In this case, the AUROC value was rectified (mirrored at 0.5) so that both negative and positive deflections resulted in values greater than 0.5. Accordingly, the choice probabilities of such a neuron were also mirrored at 0.5. We compared the choice probabilities (i.e., AUROC value for near-threshold "hit" trials vs. near-threshold "miss" trials) to a distribution of AUROC values with permuted trial labels (1000 times). A neuron was called percept related if its rectified AUROC value for near-threshold trials was greater than the 5% upper bound of the permuted distribution.

The percept-related neurons were further classified into "yes" neurons and "no" neurons according to their

neuronal activity during the selective time interval. A neuron was termed "yes" neuron if its mean firing rate was higher for "hits" in suprathreshold trials than for "correct rejections" in no-stimulus trials and if its mean firing rate was higher in near-threshold "hit" trials than in near-threshold "miss" trials. The converse relations were applied to identify "no" neurons. One neuron could not be assigned to either class because it had higher firing rates in suprathreshold "hit" than in "correct rejection" trials but lower firing rates in near-threshold "hit" than in near-threshold "miss" trials.

ROC analysis was further used to investigate whether "yes" and "no" neurons encoded the crows' later report. The choice probability index describes the AUROC value for different behavioral responses with identical stimulus properties (Britten, Newsome, Shadlen, Celebrini, & Movshon, 1996). To that aim, we used the firing rates of each neuron during its selective time interval to calculate the choice probability for "yes" versus "no" responses in near-threshold trials ("hits" vs. "misses") and in no-stimulus trials ("correct rejections" vs. "false alarms"), respectively. In addition, we calculated the AUROC value for suprathreshold "hit" versus no-stimulus "correct rejection" trials as a reference. If a neuron reflects the crow's subjective experience, it is expected to discriminate between "yes" and "no" responses, although stimulus intensities were identical, and with qualitatively similar activity as for suprathreshold "hits" versus no-stimulus "correct rejections."

To determine the onset latency and duration of significant neuronal activity for "yes" and "no" neurons, we employed a sliding window of 50-msec duration and 1-msec step size. The onset of significant neuronal activity was considered achieved when the neuronal activity differed by 3 *SD*s from the baseline over at least 26 consecutive windows. For each "yes" neuron (stimulus- and delay-related), we determined the onset and duration of significant "hit" activity in response to salient stimulus-present trials. In parallel, for each "no" neuron (stimulus- and delay-related), we determined the onset and duration of significant "correct rejection" activity in response to trials with no stimulus. A Mann–Whitney *U* test was used to compare these time values.

Neuronal activity of single cells is depicted by dot raster histograms (every line corresponds to a trial, and every dot represents an action potential) and spike density functions. Spike density functions were averaged over trials and convolved with a Gaussian kernel (bin width = 300 msec, step size = 1 msec) for illustrative purposes only.

For averaging the spike density functions of different neurons, we first normalized the firing rates by subtracting the baseline activity (firing rate in a 300-msec interval 300 msec to 0 msec before stimulus onset) and dividing by the standard deviation. The baseline activity (mean firing rate across all trials) of each neuron and its standard deviation was measured during the last 300 msec before sample onset.

To quantify the time course of information about stimulus intensity and subjective "yes" versus "no" responses, we performed a sliding-window percent explained variance (ω^2 PEV) analysis. For that purpose, we merged stimulus-related and delay-related percept-related neurons. Neurons that had sufficient trial numbers of at least 10 trials for each trial category ("hits" in suprathreshold trials, "hits" and "misses" in near-threshold trials, and "correct rejections" and "false alarms" in no-stimulus trials) entered the analysis (n = 21 "yes" neurons and 41 "no" neurons). We used a sliding window of 400-msec duration and 10-msec step size. In each window, a two-factorial ANOVA (including suprathreshold "hit," near-threshold "hit," near-threshold "miss," and no-stimulus "correct rejection" trials) was calculated, and the resultant sums of squares were used to estimate the percentage of variance attributable to either the stimulus intensity or the "yes"/"no" response for each neuron. The ω^2 was calculated as follows:

$$\omega 2 = \frac{SS_{\text{factor}} - df \times MSE}{SS_{\text{total}} + MSE}$$

where SS_{factor} is the sum of squares for the factor stimulus intensity and subjective percept ("yes"/"no" response), respectively; SS_{total} is the total sum of squares; df is the degrees of freedom, and MSE is the mean squared error. This was repeated 1000 times and then averaged. We then took the average across the individual neurons yielding a population estimate of the average percentage of variance explained by each factor.

A support vector machine (SVM) classifier was used to investigate whether the activity of a neuronal population in near-threshold trials can be used to predict the decision in supra-threshold "hit" and no-stimulus "correct rejection" trials. This was done with the same neuronal populations that were used for the PEV analysis and had at least 10 trials for each trial category ("hits" in suprathreshold trials, "hits" and "misses" in near-threshold trials, and "correct rejections" and "false alarms" in no-stimulus trials). We trained the classifier in sliding windows (400-msec length, 10-msec step size) on the firing rates of "yes" and "no" responses in near-threshold trials to exclude the factor stimulus. For each window, we used the firing rates of the neurons in 10 randomly drawn near-threshold "hit" and "miss" trials, respectively. The trained classifier was then used to predict the labels of 10 randomly drawn suprathreshold "hit" trials and 10 no-stimulus "correct rejection" trials. We calculated the percentage of "yes" predictions as a measure for decision information in the tested trials. We repeated the classifier training and prediction 1000 times with newly drawn trials and calculated the mean proportion of "yes" predictions.

RESULTS

Two carrion crows were trained in a rule-based delayed detection task to report the presence or absence of visual

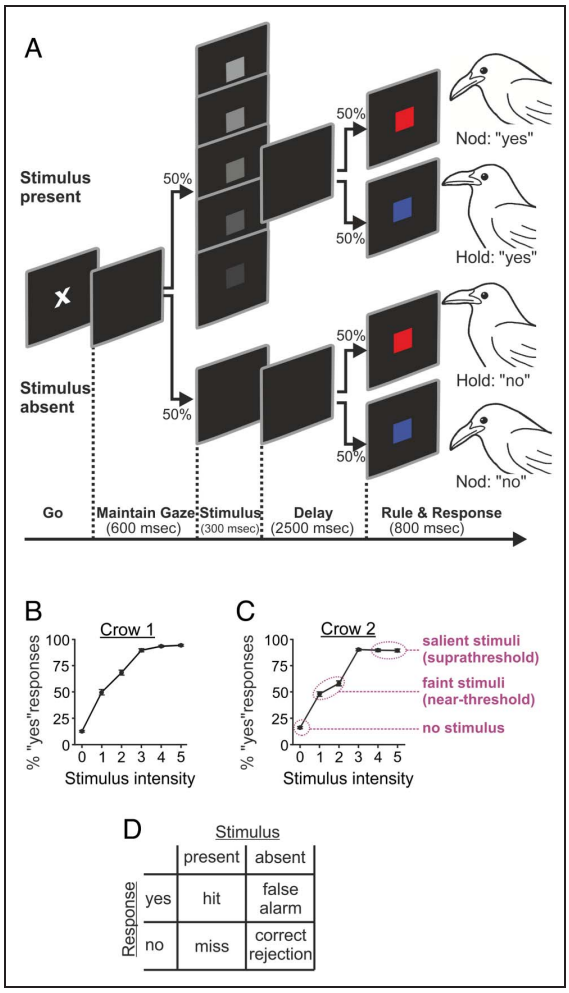

Figure 1. Task design and behavioral performance. (A) Visual detection task. After the crow initiated a trial in the go period, a brief visual stimulus of variable intensity appeared in 50% of the trials (stimulus present), whereas no stimulus appeared in the other half of the trials (stimulus absent). After a delay period, a rule cue informed the crow how to respond if it had seen or had not seen the stimulus. In stimulus trials (top), a red cue required a nodding response for stimulus detection ("yes"), whereas a blue cue required the crow to hold still for stimulus detection. In stimulus-absent trials (bottom), the rule–response contingencies were inverted. (B, C) Psychometric functions of Crow 1 (B) and Crow 2 (C). Error bars indicate *SEM*. Lilac ellipses illustrate the grouping of stimulus intensities into suprathreshold, near-threshold, and no-stimulus trials. (D) Signal detection theory classifies an observer's behavior at detection threshold, given two stimulus conditions (stimulus present or absent) and two possible responses ("yes, stimulus present" and "no, stimulus absent").

stimuli (Figure 1A). In half of the trials, a stimulus in five different intensity values around the crows' perceptual threshold (with intermediate stimulus intensities individually adjusted for each crow to result in a sigmoidal psychometric function) was presented, whereas a stimulus was absent in the other half of the trials. At perceptual threshold, the crows' conscious percept was endogenously determined; a stimulus of identical intensity was sometimes seen and other times not perceived. The crows had to wait during a delay period until a rule cue informed them about how to report their percept. Therefore, the crows were unable to prepare motor responses before the rule cues, which precluded report-related processes. This allowed us to explore neuronal activity related to subjective sensory experience and its accessibility during the delay period.

Behavior

The crows' behavioral accuracy (percent correct "yes" responses) was plotted as a function of stimulus intensity to result in a classical psychometric function (Figure 1B and C). Depending on the crows' accuracy, the trials were grouped into three categories: suprathreshold trials (presenting the two highest stimulus intensities), near-threshold trials (in which the two lowest stimulus intensities at perceptual threshold of about 50% hit rate were shown), and no-stimulus trials (without any stimulus shown; Figure 1C). The crows' responses were classified according to the framework of signal detection theory (Green & Swets, 1966): "hit" (correct "yes" response to a stimulus), "correct rejection" (correct "no" response for stimulus absence), "miss" (erroneous "no" response to stimulus presence), and "false alarm" (erroneous "yes" response for stimulus absence; Figure 1D). These response categories were later used to classify and compare neuronal activity during task performance.

Neurophysiology

We recorded action potentials from a total of 480 neurons ($n = 174$ for Crow 1, $n = 306$ for Crow 2) in the NCL of the crows while they performed the task (Figure 1A; see Nieder et al., 2020). On the basis of a sliding-window analysis comparing firing rates for suprathreshold "hit" trials versus no-stimulus "correct rejection" trials in individual neurons (Mann–Whitney U test, $p < .01$), we first isolated 262 task-selective neurons that showed selective trial intervals at some point during the stimulus and/or delay phase. According to the two most important trial phases in which report-independent subjective experiences about the stimulus situation occurred, we classified task-selective neurons into stimulus related ($n = 155$) and delay related ($n = 165$). Most neurons showed transient task-selective epochs but, as a population, spanned the entire trial period until rule cue presentation (see Figure 2A and B in Nieder et al., 2020).

Task-selective neurons may simply respond to the different intensities of the stimulus. To identify neurons that changed activity as a function of the crows' percept as reported later in the trial (later called "percept-related neurons"), we compared the discharges during the crows' "yes" versus "no" responses in near-threshold trials. If

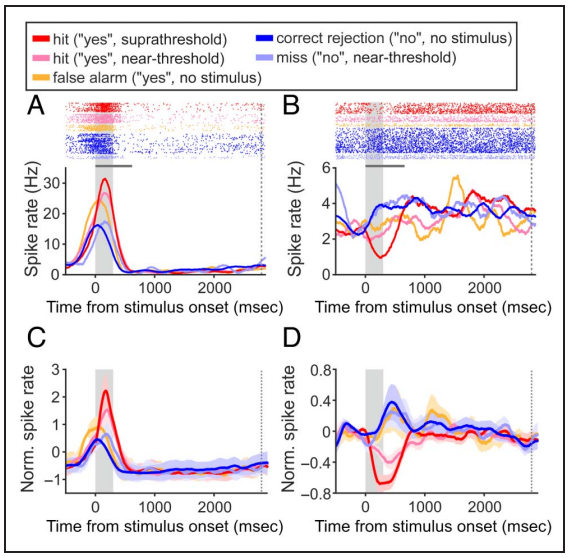

Figure 2. Activity of percept-related NCL neurons during stimulus presentation. (A) Activity of an example "yes" neuron. The top depicts dot raster histogram with each line corresponding to a trial and each dot corresponding to an action potential. The bottom represents the respective averaged spike density functions (smoothed by a 300-msec Gaussian kernel with a step size of 1 msec). Each curve corresponds to one of the five trial categories with warm (red, pink, and orange) colors indicating "yes" responses and cool (dark and light blue) colors indicating "no" responses. The gray-shaded area indicates stimulus presentation time; and the dashed vertical line, the end of the delay period (onset of response rule cue). The horizontal gray bar signifies the task-selective interval. (B) Activity of an example "no" neuron. Layout as in A. (C) Averaged, normalized activity of the population of all "yes" neurons (n = 3). Shaded regions indicate *SEM*. Color code and layout as in A. (D) Averaged, normalized activity of the population of all "no" neurons (n = 17). Shaded regions indicate *SEM*. Color code and layout as in A. Norm. = Normalized.

neurons are percept related and represent the crows' reported subjective experience, they are expected to change activity as a function of the crows' later report and irrespective of the identical stimulus intensity. In this case, firing rates in near-threshold trials during the crows' "yes" responses ("hits") should be similar to those during "yes" responses ("hits") in suprathreshold trials. In contrast, firing rates of percept-related neurons in near-threshold trials during the crows' "no" responses ("misses") should be similar to those during "no" responses ("correct rejections") in no-stimulus trials. Firing rates to "false alarms" were not included as additional criterion for the selection of percept-related neurons because of activity noise caused by low trial counts. However, "false alarms" were analyzed qualitatively for the selected percept-related neurons.

Activity of Percept-related Neurons

To objectively identify percept-related neurons, we applied the following statistical criteria in unison: First, firing rates in suprathreshold "hit" compared to no-stimulus "correct rejection" trials had to be significantly different ("task-selective neuron"; Mann–Whitney U test, $p < .01$). Second, we performed an ROC analysis (i.e., a binary classifier) with firing rates taken from the selective trial intervals during "yes" versus "no" responses in near-threshold trials as well as for stimulus and delay periods separately. We derived the AUROCs as a distribution-free discriminability measure (Green & Swets, 1966). AUROC values ("choice probabilities") comparing near-threshold "hit" trials versus near-threshold "miss" trials had to be significantly different from 0.5 for percept-related neurons (permutation test, 1000 shuffled distributions, $p < .05$).

Neurons that met both criteria were classified into "yes" neurons if firing rates to "yes" responses were higher compared to "no" responses or "no" neurons if firing rates to "no" responses were higher compared to "yes" responses. Moreover, for a neuron to be classified as percept-related "yes" or "no" neuron, the firing rate changes for both comparisons had to concur for "yes" versus "no" responses; in other words, if a neuron increased its firing rate to suprathreshold "hits," it also had to increase its firing rate to near-threshold "hits" to be classified as a "yes" neuron, and vice versa for "no" neuron.

During the stimulus presentation phase, we found that 14% of the task-related neurons (21/155) were percept related (Table 1). Of those, three percept-related neurons showed higher firing rates to "yes" percepts (stimulus-present percept) compared to "no" percepts and were called "yes neurons." In contrast, 17 percept-related neurons exhibited higher firing rates to "no" percepts (stimulus-absent percept) compared to "yes" percepts and were called "no neurons." One neuron could not be assigned to either class. Two percept-related example neurons during the stimulus presentation period are shown in Figure 2A and B. Both neurons signaled the "yes" versus "no" percepts later reported by the crows and irrespective of the stimulus intensity in the different trial conditions. However, whereas the neuron in Figure 2A was a "yes" neuron and increased its firing rate for "yes" percepts, the neuron

Table 1. Number of "Yes" and "No" Neurons Among the Percept-related Neurons

	Percept Related	*"Yes" Neurons*	*"No" Neurons*	*Not Determinable*
Stimulus related	21	3	17	1
Delay related	47	19	28	

in Figure 2B qualified as a "no" neuron because it increased its firing rate to "no" percepts. This pattern of activation in example neurons was seen for the population of significant "yes" neurons (Figure 2C) and "no" neurons (Figure 2D).

During the delay phase, a significantly higher proportion of 28% (47/165) of the percept-related neurons was identified compared to the stimulus presentation period (chi-square test; $p = .001$). Here, more balanced numbers of 19 "yes" neurons and 28 "no" neurons were detected. Two percept-related example neurons during the delay period are shown in Figure 3A and B. Both neurons signaled the "yes" versus no" percepts irrespective of the stimulus intensity in the different trial conditions. The neuron in Figure 3A increased its firing rate for "yes" percepts and was classified as a "yes" neuron. In contrast, the neuron in Figure 3B increased its firing rate to "no" percepts and qualified as a "no" neuron. Activation during "false alarms" ("yes" percepts) was more similar to suprathreshold and near-threshold "hits," although no stimulus was presented. Similar patterns of overall activation as for the example neurons were seen for the population of significant "yes" neurons (Figure 3C) and "no" neurons (Figure 3D).

The negative deflection of activity for "hits" in "no" neurons (Figure 2B and D) could result from two different conditions: The deflection could reflect suppression below the neurons' spontaneous activity; alternatively, neuronal firing could assume an increased tonic state with trial onset that is then switched off by stimulus appearance. We, therefore, compared the firing rates of individual neurons before the start of the trial with their activity after the onset of the trial but before stimulus appearance. If the neurons assume an elevated tonic state with trial onset, the firing rate is expected to be higher in the period before stimulus onset that we defined as baseline activity. We found that the firing rate in a 300-msec period before the start of a trial (400 msec to 100 msec before trial onset) was 5.68 Hz on average and indifferent from baseline activity of 5.59 Hz determined before the presentation of the stimulus (300 msec to 0 msec before stimulus onset; Wilcoxon signed-rank test, $p = .6617$, $n = 66$). This suggests that the neurons did not increase their firing rates to assume an increased tonic state in response to the absence of a stimulus. Rather, the suppression with perceived stimulus onset observed in "no" neurons (Figure 2B and D) reflects suppression below spontaneous activity in these neurons.

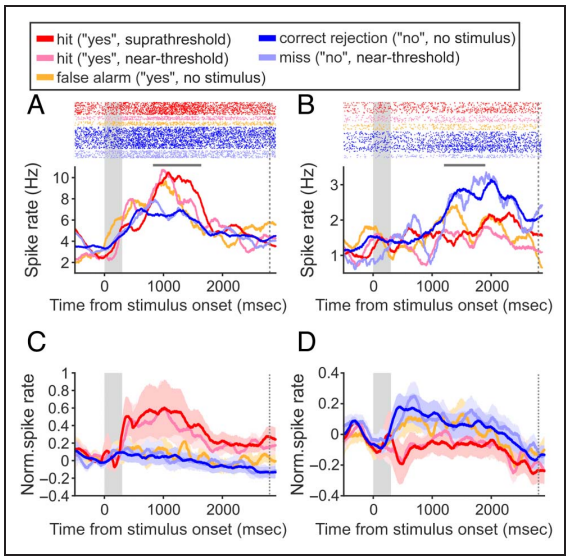

Figure 3. Activity of percept-related NCL neurons in the delay period. (A) Activity of an example "yes" neuron. Color code and layout as in Figure 2A. (B) Activity of an example "no" neuron. Color code and layout as in Figure 2A. (C) Averaged, normalized activity of the population of all "yes" neurons ($n = 19$). Shaded regions indicate *SEM*. Color code and layout as in A. (D) Averaged, normalized activity of the population of all "no" neurons ($n = 28$). Shaded regions indicate *SEM*. Color code and layout as in A. Norm. = Normalized.

Next, we explored potential differences in onset latency of "yes" and "no" neurons. For each "yes" neuron (stimulus- and delay-related), we determined the onset and duration of significant "hit" activity in response to salient stimulus-present trials. Similarly, for each "no" neuron (stimulus- and delay-related), we determined the onset and duration of significant "correct rejection" activity in response to trials with no stimulus. We found that the onset latency of "yes" neurons (mean = 179 msec) was significantly shorter compared to the onset latency of "no" neurons (408 msec; Mann–Whitney *U* test, two-tailed, $p = .0014$). No difference was detected for the duration of significant response intervals between both neuron types (Mann–Whitney *U* test, two-tailed, $p = .1211$).

Choice Probabilities

To quantify how well neurons discriminated the behaviorally relevant "yes" and "no" percepts irrespective of stimulus intensity, we calculated AUROC values for "yes" versus "no" responses (termed "choice probabilities"). To that aim, we compared the firing rates in near-threshold "hit" versus "miss" trials as well as "correct rejection" versus "false alarm" trials. As a reference, we also calculated the AUROC value for suprathreshold "hit" versus no-stimulus "correct rejection" trials. AUROC values of "no" neurons were, by definition, smaller than 0.5 and were rectified for further analysis. Choice probabilities were then assessed separately for percept-related neurons in the stimulus and delay periods. The choice probabilities (gray columns in Figure 4) were plotted relative to the reference AUROC values (suprathreshold "hit" vs. no-stimulus "correct rejection" trials; black columns in Figure 4).

In the stimulus presentation period, the reference AUROC values ("hits" in suprathreshold trials vs. "correct rejections") were 0.82 for "yes" neurons (Figure 4A and C)

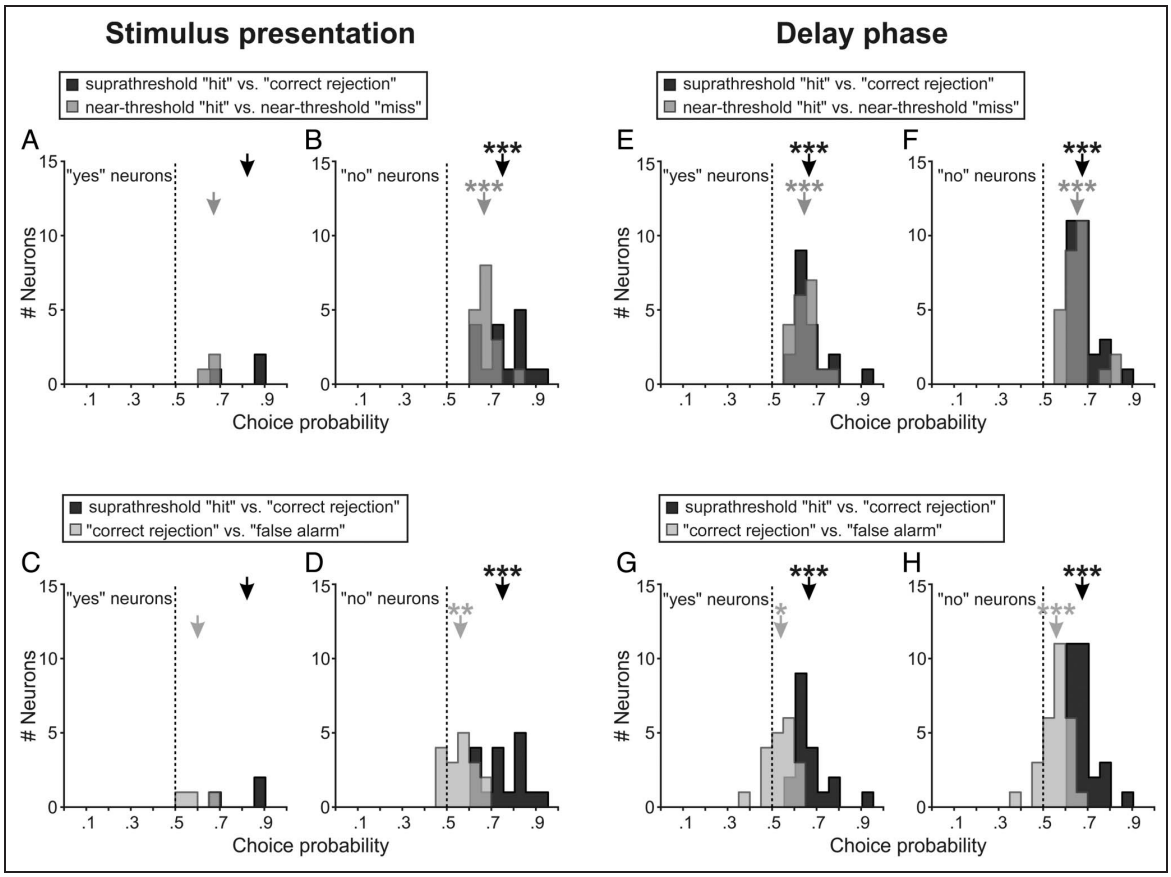

Figure 4. Choice probabilities of percept-related neurons. (A–D) Choice probabilities for percept-related neurons during stimulus presentation. (A) Reference AUROC values of "yes" neurons and their choice probabilities for near-threshold "hits" versus near-threshold "misses." Arrows indicate mean choice probabilities. Vertical dashed line depicts chance level at 0.5. (B) Reference AUROC values of "no" neurons and their choice probabilities for near-threshold "hits" versus near-threshold "misses." Asterisks indicate significant difference to chance level (one-sample Wilcoxon signed-rank test; ***$p < .001$, **$p < .01$, *$p < .05$). (C) Reference AUROC values of "yes" neurons and their choice probabilities for "correct rejections" versus "false alarms." (D) Reference AUROC values of "no" neurons and their choice probabilities for "correct rejections" versus "false alarms." (E–H) Choice probabilities for percept-related neurons during the delay phase. Same layout and trial conditions as in A–D.

and 0.75 for "no" neurons (Figure 4B and D), respectively, and were indifferent (Mann–Whitney U test, $p = .24$). The reference ROC values of the "no" neurons were significantly higher than chance level (one-sample Wilcoxon signed-rank test, $p < .0003$, $n = 17$). The "yes" neurons could not be tested because of the low number of only three neurons. In addition, because of the low neuron numbers, the choice probabilities of the few "yes" neurons in the near-threshold "hit" versus near-threshold "miss" trials (mean = 0.67; Figure 4A) and the "correct rejection" versus "false alarm" trials (mean = 0.60; Figure 4C) were indifferent from chance level of 0.5 (one-sample Wilcoxon signed-rank test, $p = .25$, $n = 3$). However, the choice probabilities of the "no" neurons were significantly higher than the chance level of 0.5 in the near-threshold "hit" versus "miss" trials (mean = 0.67, one-sample Wilcoxon signed-rank test, $p < .0003$, $n = 17$; Figure 4B) and also for "correct rejection" versus "false alarm" trials (mean = 0.56, one-sample Wilcoxon signed-rank test, $p < .0057$, $n = 17$; Figure 4D).

In the delay period, the reference AUROC values of 0.67 for "yes" neurons (Figure 4E and G) and 0.68 for "no" neurons (Figure 4F and H), respectively, were indifferent (Mann–Whitney U test, $p = .22$) but significantly above chance (one-sample Wilcoxon signed-rank test, both ps $< .0002$, $n = 28$ "yes" neurons and 19 "no" neurons). Furthermore, in the delay period, the choice probabilities of "yes" and "no" neurons in the near-threshold "hit" versus near-threshold "miss" trials were both significantly higher than chance (one-sample Wilcoxon signed-rank test, both ps $< .0002$, $n = 28$ "yes" neurons and 19 "no" neurons) and indifferent, with means of 0.64 and 0.65, respectively (Mann–Whitney U test, $p = .74$; Figure 4E and F). Moreover, the choice probabilities of "yes" and "no" neurons in the "correct rejection" versus "false alarm" trials had similar means of 0.54 and 0.56, respectively

(Mann–Whitney U test, $p = .23$), and were both higher than chance (one-sample Wilcoxon signed-rank test, "yes" neurons: $p < .013$, $n = 19$; "no" neurons: $p < .0002$, $n = 28$; Figure 4G and H). Taken together, both "yes" and "no" neurons in the delay period encoded the crows' subjective percept irrespective of stimulus intensity in ambiguous trials. A higher proportion of neurons turned out to be percept related during the delay period compared to the stimulus presentation period, and "yes" and "no" neurons were more balanced during the delay period than during the stimulus presentation period.

Neuron Population Analyses

Next, we quantified how much information about the subjective report as opposed to stimulus intensity was carried by the separate populations of "yes" and "no" neurons throughout the trial. To that aim, we merged the percept-related neurons with sufficient trial numbers in the stimulus and delay periods and calculated the ω^2 PEV in sliding windows throughout the trial. For the population of "yes" neurons ($n = 21$), the information about stimulus intensity and subjective experience oscillated until the end of the delay. Briefly before the onset of the response rule, information about stimulus intensity had vanished, whereas subjective experience information increased notably shortly before the crows reported their percept (Figure 5A). For "no" neurons ($n = 41$), the presentation of the stimulus elicited a sharp increase of information about the stimulus intensity, followed by a slightly delayed increase of information about the subjective report (Figure 5B). After a decay during the first half of the delay, information about the subjective report increased again toward the end of the delay, whereas stimulus information had vanished.

Finally, we tested with a decoding analysis whether the subjective report in suprathreshold "hit" and no-stimulus "correct rejection" trials can be predicted separately by "yes" and "no" neurons in near-threshold trials. Assuming that a percept-related neuron encodes the subjective report, its firing rates should be similar according to the subjective experience and thus predictive of the report irrespective of the stimulus intensity. To investigate this, we trained an SVM classifier using the same separate neuronal populations as before ($n = 21$ "yes" neurons and 41 "no" neurons). We trained the classifier on firing rates of "yes" and "no" responses in near-threshold trials and then tested it on the firing rates in suprathreshold "hit" and no-stimulus "correct rejection" trials of the same neuronal population.

On the basis of the population of "yes" neurons, the classifier labeled "yes" and "no" responses with highest accuracy shortly after stimulus presentation and at the end of the delay (Figure 6A). At these time points, the difference between "yes" predictions to firing rates in suprathreshold "hit" and no-stimulus "correct rejection" trials was greatest (Figure 6B). In addition, training the classifier on the activity of the population of "no" neurons in near-threshold trials yielded the highest prediction accuracy for "yes" responses in suprathreshold "hit" and no-stimulus "correct rejection" trials shortly after stimulus presentation and, after a drop-off, increasingly in the second half of the delay (Figure 6C). Apart from more pronounced accuracy after stimulus onset, the time course of the accuracy of "yes" predictions was comparable for "yes" neurons (Figure 6B) and "no" neurons (Figure 6D).

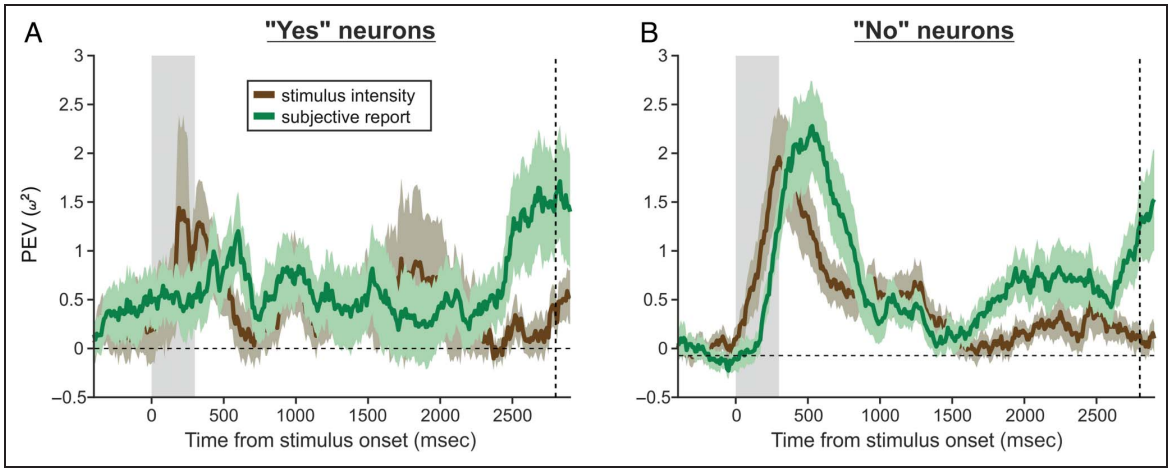

Figure 5. Time-resolved PEV analysis. (A) Time course of information about stimulus intensity and subjective report carried by the activity of "yes" neurons ($n = 21$) throughout a trial. Colored shadings indicate *SEM* across the neurons. Gray-shaded area depicts stimulus presentation time; and vertical dashed line, the end of the delay (onset of response rule cue). (B) Same as in A but for the population of "no" neurons ($n = 41$).

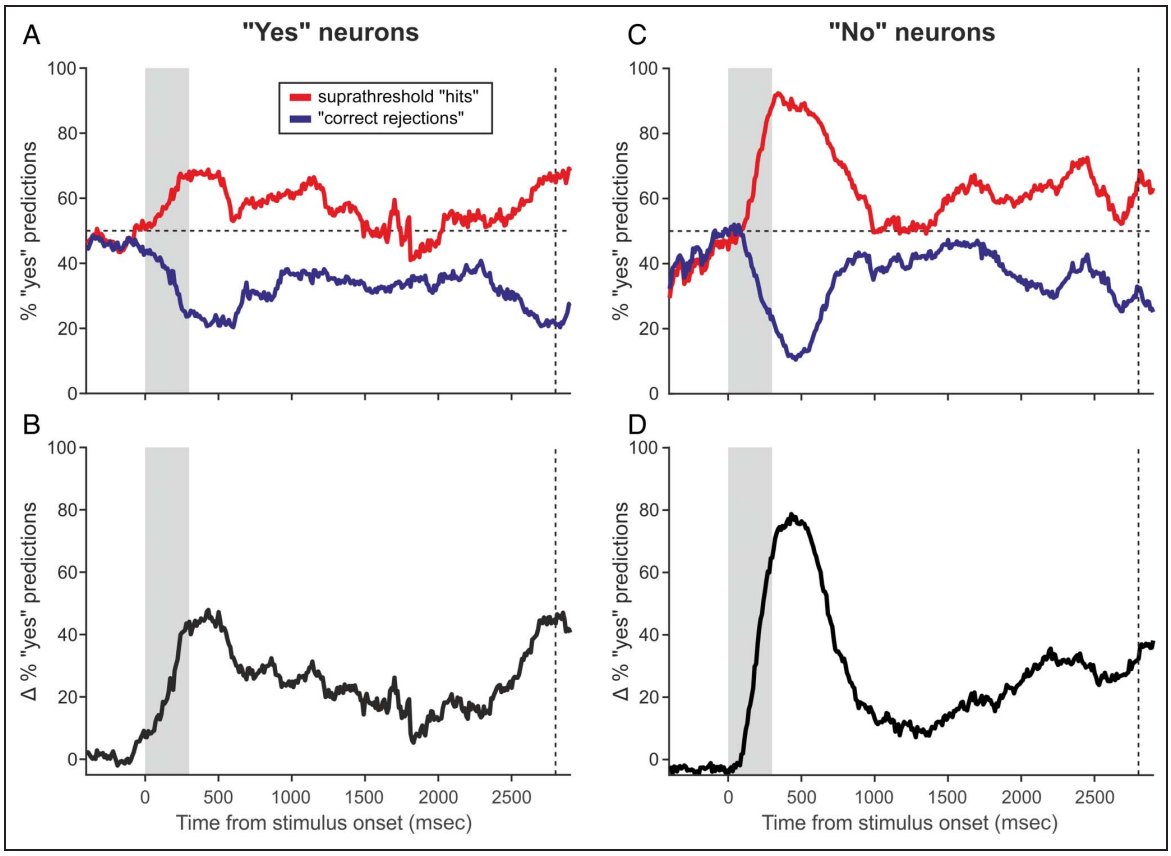

Figure 6. Classification accuracy of an SVM classifier. (A) Proportion of assigned "yes" labels to firing rates of "yes" neurons (n = 21) in suprathreshold "hit" trials and no-stimulus "correct rejection" trials of an SVM classifier, which was trained on the firing rates of "yes" and "no" responses in near-threshold trials. Horizontal dashed line indicates chance level at 50%. Gray-shaded area depicts stimulus presentation time, and vertical dashed line indicates end of the delay (onset of response rule cue). (B) Difference between the proportions of assigned "yes" labels to suprathreshold "hit" and no-stimulus "correct rejection" trials shown in A. (C) Same as in A but for the population of "no" neurons (n = 41). (D) Same as in B but for the population of "no" neurons (n = 41).

DISCUSSION

We trained crows to report the presence or absence of low-contrast images presented near to visual threshold (note that this process only requires awareness; it does not require self-awareness or metacognition of experience). Neuronal activity tracking the conscious percept (i.e., present or absent) was dissociated from that involved in planning a motor response by use of a poststimulus response rule cue that varied from trial to trial. Distinct populations of "yes" and "no" neurons signaled the subjective percepts of "seen" versus "unseen," respectively. Importantly, the magnitude of activation of these two neuron populations was similar in timing and strength. This suggests a balanced encoding of awareness in the crow NCL by neurons actively signaling subjective stimulus presence and absence.

The shorter onset latency of "yes" neurons compared to "no" neurons suggests that the neuronal activation of "yes" neurons to the onset of a seen stimulus was temporally more precise and thus faster compared to the activation related to stimulus absence in "no" neurons. For "no" neurons, responses to the absence of a stimulus may be less precise and potentially more variable from trial to trial (Ganupuru, Goldring, Harun, & Hanks, 2019).

Active Encoding of Percepts About Stimulus Absence in the Brain

A main finding of the current study is that a distinct population of "no" neurons in the crow NCL actively encoded the crows' perceived absence of stimuli by firing rate increases. This is a remarkable finding as it is commonly assumed that only the perceived presence of stimuli is actively encoded by increasing firing rates of neurons (de Lafuente & Romo, 2005, 2006). According to this common assumption, only the conscious presence of a stimulus is signaled by neurons that accumulate positive stimulus evidence until an upper threshold is reached that

causes a conscious stimulus-present percept; the conscious no-stimulus percept is supposed to be represented by the absence of specific neuronal activity equivalent to resting state activity (Pereira et al., 2022).

In line with our finding, previous studies reported that a behaviorally relevant lack of sensory evidence favoring perceived absence of a stimulus may also be actively encoded by neurons in cortical association areas of nonhuman primates, animals known to show visual awareness (Ben-Haim et al., 2021). Neurons in the dorsolateral pFC of macaque monkeys reporting the subjective presence or absence of visual stimuli actively signal the perceived absence of a stimulus (Merten & Nieder, 2012). Such stimulus-absence signals in pFC are predominantly found during the delay period after a missed stimulus (Merten & Nieder, 2012). Similar findings were reported in single-neuron recordings in posterior parietal cortex of human patients with epilepsy while they detected weak and unpredictable vibrotactile stimuli (Pereira et al., 2021). In this human study, some neurons showed a higher increase in firing rates for misses compared to hits, raising the intriguing possibility that missed/absent percepts are encoded actively also in the human brain (Pereira et al., 2021). These empirical findings agree with models of awareness states that postulate symmetric/balanced encoding of presence and absence experiences (Fleming, 2020, 2021). Together, these data call for a greater focus on examining percepts and decisions about stimulus absence. These findings also question whether absence percepts can be used as a baseline or control condition in studies of perceptual awareness, as is often done.

Temporal Two-stage Process of Sensory Consciousness

Our results in crows suggest a temporal two-stage process in sensory consciousness. NCL "yes"- and "no"-neuron populations change from initially predominantly encoding stimulus intensity to mainly representing the crows' subjective experience later in the trial and before a behavioral report is required. Notably, the active coding of the "stimulus absence" percept primarily emerged during the delay phase when the crows' subjective percept was maintained until the response type was instructed. This suggests a postsensory, cognitive processing stage in which the categorical "no" signal arose.

This activation cascade is reminiscent to results in the primate cerebral cortex; here, the early activity is also primarily involved in unconscious vision, whereas neuronal responses associated with subjective experiences are delayed relative to stimulus onset (Quiroga et al., 2008; de Lafuente & Romo, 2006; Supèr, Spekreijse, & Lamme, 2001; Lamme & Roelfsema, 2000; Thompson & Schall, 1999). This two-stage process in conscious perception may constitute a general principle of how sensory awareness is realized in the vertebrate brain.

The two-stage process can, in principle, be explained by the "global neuronal workspace theory" (Dehaene & Changeux, 2011; Baars, 2002). This neurobiological conception of consciousness theorizes that only intensive enough sensory activity is able to access awareness by eliciting a network state called "global ignition" in higher brain centers such as the primate pFC. The NCL would be the ideal site for such an "ignition" because—like pFC in the primate brain—it operates at the apex of the telencephalic processing hierarchy in the avian brain (Nieder, 2017; Güntürkün, 2005). This "all-or-none ignition" event results in stimulus-driven activity to become persistent in recurrent and interconnected brain networks, even after the stimulus itself has vanished (Mashour, Roelfsema, Changeux, & Dehaene, 2020; van Vugt et al., 2018). This can explain why percept-related activity in NCL is seen in the delay phase after the brief stimulus has ceased.

As an elaboration and extension of the original "global neuronal workspace theory," our findings suggest that sensed stimulus energy is not the only trigger that can lead to an ignition of large-scale networks when causing "stimulus presence" percepts. Rather, the absence of stimuli can also ignite brain networks by sufficient activation of pools of "no" neurons to cause explicit "stimulus absence" experiences, as long as "nothing" is a behaviorally relevant category. As "no" neurons cannot be excited by incoming stimulus energy (which is lacking by definition for absent stimuli), brain-internal mechanisms must excite (or disinhibit) "no" neurons to signal conscious "absence" states as subjective categorical representation. The precise mechanisms of how "no" neurons become activated needs to be deciphered in the future.

"Nothing" Represented as a Behavioral Category

In our behavioral protocol, not only the presence but also the absence of stimuli was behaviorally relevant and needed to be reported by the crows. Therefore, "nothing" became a behavioral category and as such was most likely needed to be actively encoded by neurons. This categorical active "absence" signal is reminiscent of quantitative empty-set representations (Nieder, 2016). Neurons in the crow (Kirschhock, Ditz, & Nieder, 2021) and monkey brain (Ramirez-Cardenas & Nieder, 2019; Ramirez-Cardenas, Moskaleva, & Nieder, 2016; Okuyama, Kuki, & Mushiake, 2015) are tuned to the preferred numerosity zero (i.e., the empty set). Numerosity-zero-tuned neurons respond with a maximum discharge to numerosity zero and show a progressive drop-off of activity toward higher numerosities. Neurons tuned to zero even emerge spontaneously in deep neural networks of object discrimination (Nasr & Nieder, 2021).

Both "stimulus absence" and "empty set" activity require a transformation from a sensory "no-event" to an internally generated, categorical representation, probably through trial-and-error reinforcement learning. A cortical

circuit model exemplified how category selectivity could arise from reinforcement learning (Engel, Chaisangmongkon, Freedman, & Wang, 2015). This model posits that systematic correlations between trial-to-trial fluctuations of firing rates and the accompanying reward after appropriate behavioral choices cause neurons that progressively become category selective (Engel et al., 2015). According to this model, even initially nonselective neurons developed categorical tuning, as long as they exhibit firing rate fluctuations that correlated with behavioral choices. Thus, when a crow learns to explicitly respond to "nothing" or numerosity zero to receive a reward, this mechanism might suffice to produce neurons that respond actively to "no" percepts and numerical zero categories.

Neurobiological Principles of Sensory Consciousness Across Evolution

Our findings in crows can also inform the neurobiological principles of sensory consciousness across evolution (Nieder, 2022; Nieder et al., 2020). Birds diverged from the mammalian lineage 320 million years ago (Hedges, 2002; Kumar & Hedges, 1998). Since then, birds evolved radically different endbrain structures (Jarvis et al., 2005). Nevertheless, some birds, notably members of the corvid songbird family (crows, ravens, jays), show sophisticated cognitive behaviors such as endogenous attention (Hahner & Nieder, 2023; Quest, Rinnert, Hahner, & Nieder, 2022) and robust working memory (Wagener, Rinnert, Veit, & Nieder, 2023; Liao, Brecht, Johnston, & Nieder, 2022; Smirnova, Zorina, Obozova, & Wasserman, 2015; Veit & Nieder, 2013) indicative of conscious experiences (Nieder, 2022, 2023; Nieder et al., 2020). In contrast to mammals, the crow telencephalon—and the NCL in particular—is lacking a layered neocortex and has instead evolved a nuclear anatomical arrangement with surprisingly high associative neuron numbers (Kersten et al., 2022; Ströckens et al., 2022; Olkowicz et al., 2016). Our data suggest that the active coding of both stimulus presence and absence is a computational principle for sensory consciousness irrespective of the precise anatomical layout and across remotely related phylogenetic taxa (Nieder, 2021).

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Data Availability Statement

Code is available from the corresponding author upon reasonable request. All behavioral and electrophysiological data are archived at the Institute of Neurobiology, University of Tübingen, Germany.

Author Contributions

A. N. and L. W. designed and conducted the experiments, analyzed the data, and wrote the article. A. N. supervised the study.

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Diversity in Citation Practices

Retrospective analysis of the citations in every article published in this journal from 2010 to 2021 reveals a persistent pattern of gender imbalance: Although the proportions of authorship teams (categorized by estimated gender identification of first author/last author) publishing in the *Journal of Cognitive Neuroscience* (*JoCN*) during this period were M(an)/M = .407, W(oman)/M = .32, M/W = .115, and W/W = .159, the comparable proportions for the articles that these authorship teams cited were M/M = .549, W/M = .257, M/W = .109, and W/W = .085 (Postle and Fulvio, *JoCN*, 34:1, pp. 1 -3). Consequently, *JoCN* encourages all authors to consider gender balance explicitly when selecting which articles to cite and gives them the opportunity to report their article's gender citation balance.

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