Processing of spatial information during goal directed behavior in the carrion crow endbrain

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Abstract

Goal directed behavior, as opposed to habitual or conditioned behavior, is believed or known to have a causal effect that brings the agent closer to a goal. In order to be able to perform goal directed behavior, at least these three steps of processing are necessary: first, sensory information must be integrated, maintained and analyzed for possible behavioral response. This maintenance and analysis of information takes place in working memory. Secondly, as a result of the analysis in working memory, the motor response can be planned. Finally, the motor plan is executed, actualizing the goal directed behavior.

This thesis investigates the involvement of the avian endbrain area *Nidopallium caudolateral*e (NCL) in processing of spatial information for goal directed behavior. We trained carrion crows (*Corvus corone*) on two different behavioral protocols, in which they either had to remember the spatial location of an object or prepare a movement to a spatial location. While the animals were engaged in these protocols, we recorded the activity of single neurons in the NCL. Single neurons in NCL selectively increased their firing rates during visual presentation of different spatial locations, encoding the spatial sensory input. In addition, NCL neurons also maintained this selectivity over a memory delay in the absence of visual stimulation. Similar maintained selectivity was previously interpreted as a correlate for working memory. Another population of NCL neurons selectively encoded the location of a future motor response, while the crows were planning the movement. Finally, NCL neurons represented the movement direction during execution of the behavioral response. These findings demonstrate that NCL is involved in sensory encoding and maintenance of spatial information in working memory. In addition, NCL also takes part in planning of motor responses and encodes the direction of motor response during execution.

Our findings parallel previous findings in the mammalian prefrontal cortex (PFC) which is also involved in both spatial working memory and motor planning. Although birds and mammals show drastically different brain architectures, the avian NCL and mammalian PFC are astoundingly similar in the processing of spatial information. This thesis emphasizes the previously proposed functional analogy between NCL and PFC.

Part I.
Synopsis

1. Introduction

Spatial information describes the location of subjects and objects but can also be attributed to movements and directions. Due to its elementary nature, spatial information is ubiquitous and plays a constant role in our everyday life: *Where are my keys? Where is Waldo? Where are you going? Where will I put this box?* We automatically attribute everything that we can interact with or think of with spatial information. Often the absence or missing of spatial information alerts us and causes behavioral and cognitive processes that are aimed to resolve that lack of information. Due to its omnipresence, spatial information is an ideal feature to investigate the stages of information processing ranging from intake of sensory information to the behavioral output.

Behavior that is executed in order to achieve a goal, can be subsumed under the term goal directed behavior. Goal directed behavior also includes behaviors that are not directly resulting in the favored outcome but are intended to bring the organism closer to it. In order to filter the vast sensory input and compare available information with the inherent goals, an organism needs control functions that efficiently allocate the limited resources that are available. This managing of resources and selecting of information for processing is thought to be accomplished by so-called executive control. Executive control functions are located at the apex of the processing hierarchy in association areas that are reciprocally connected to sensory input and motor output areas. In these association areas, information from multiple sensory modalities can be processed and integrated with previous and internal information. Their reciprocal connection to sensory as well as motor areas, enables them to subsequently influence both sensory pre-processing and motor behavior.

In this thesis, I present data that show the representation of spatial information during different steps of goal directed behavior in an association area in the crow endbrain. Focusing on spatial information, I look at different stages of information processing, starting at the encoding of sensory information, then progressing to the maintenance of that information for later use, and finally analyzing the planning of a spatial response and the execution of that response toward a target location.

1.1. A simplified model for information processing for goal directed behavior

In a very liberal definition, goal directed behavior comprises all actions that are executed with the intent of bringing an organism in the direction of a goal. A more precise definition was proposed by de Wit and Dickinson (2009). According to their definition, goal directed behavior has to be instrumental in contrast to behavior that is habitual. As a first requirement instrumental behavior is learned and not innate. In addition, the acting agent must have an understanding or belief that the specific action will have a certain effect. This causal knowledge of action and outcome is in contrast to a habitual action that is purely stimulus-driven (e.g. a Pavlovian conditioned response). As a final requirement, the agent has to desire the outcome that its action will yield. In other words, the outcome has to be a goal of the agent. This is again in contrast to a Pavlovian response, that is also executed when the outcome is not desired at the time. Hence, goal directed behavior implies a complex set of cognitive processes, because different behavioral possibilities have to be compared and analyzed to achieve the internally represented goal. On a simplified level, goal directed behavior can be split up in three steps of processing, which I will elaborate in the following.

In the first step, information from various sources has to be integrated and analyzed with respect to an internal goal. Current sensory information is combined with the internal status and compared to previous information from long term memory and analyzed for possible behavioral responses. These responses must then be evaluated for their effects on an internal goal. In order to allow integration and analysis of information from different sources, the information must be maintained for at least a short period of time. This maintenance and integration of information is thought to take place in so-called working memory, one core component of executive function (Baddeley, 2003).

Following the initial integration of information in working memory, the second step toward a goal directed response is the selection and planning of a motor response. The motor plan contains two components: the action plan and the point in time for execution. The action plan covers the actual movement (e.g. *grab object X with the right hand*). While the time for execution can be immediate, it is often beneficial to delay a motor response for either a fixed time or link it to an event (e.g. *execute action Y when event Z occurs*). Both the decision and the planning of a response are per definition cognitive processes separate from working memory (Fuster, 2001; Baddeley, 2003), however they are closely linked and dependent on each other. Irrespective of the affiliation of motor planning and working memory, planning is a complex cognitive process that is part of the set of executive control functions.

In the third and final step, the planned action is transformed into a motor response, realizing the actual goal directed behavioral output. In this simplified description of goal directed behavior, the execution of the motor response completes the perception-action cycle and would be followed by the next cycle. Although the execution of a motor response is finally carried out in less cognitive motor areas, the execution of movements must be constantly monitored to allow for adjustments in the motor response (Fuster, 2017).

In summary, three steps of processing information are necessary for goal directed behavior: First, sensory information needs to be maintained and analyzed in working memory. Second, a goal directed response has to be chosen and planned. In the third step, the planned response is executed, realizing the goal directed behavior

1.2. Why investigate cognitive processes in corvids?

When most people think of animals with exceptional cognitive abilities, they think of their own species – the human – in a first step and in a second step of our close relatives, the apes and monkeys. Humans possess, due to being the sole investigator of cognition, all cognitive skills known to exist and are the benchmark for cognitive abilities in other species. Also, nonhuman primates were shown to have a set of cognitive abilities that they share with us, suggesting a common set of basic cognitive tools. This set includes aspects of using and manufacturing tools, understanding of causality, future planning, theory of mind, and many others (Seed and Tomasello, 2010). For a long time, the cognitive hierarchy with humans at the top followed by nonhuman primates seemed unchallenged. It was only in recent years that more and more studies reported complex cognitive abilities in species that were only distantly related: birds. The class *Aves*, especially parrots and corvids, has demonstrated astonishing behavior that suggest that their cognitive abilities are on par with nonhuman primates (Emery and Clayton, 2004; Clayton and Emery, 2015). In the following I will review previous studies on corvids, revealing their comprehensive cognitive repertoire.

Corvids use tools in order to reach objects that they would otherwise not be able to obtain. New Caledonian crows are famous for both using and manufacturing of two different kind of tools. These tools are used to extract prey insects from dead wood or other hard to reach places. Interestingly, the crows have at least two different types of tools, that are suggested to be used for different tasks (Hunt, 1996). Perhaps the most famous individual corvid was New Caledonian crow Betty, that, in an experiment, bent metal wire, a material that was novel to the bird, to create a hook tool in order to retrieve a food reward (Weir et al., 2002). Tool use is also found in other corvid species, like the only distantly related Hawaiian crows (Rutz et al., 2016; Uomini and Hunt, 2017). Finally, Bird and Emery (2009) reported that also usually non tool using rooks, spontaneously used tools in an experiment. Similar to the New Caledonian crow Betty, these rooks bend wire to retrieve food from a tube. The latter experiment suggests that all corvids might have a capability to solve problems flexibly, for example, by creating a tool (Bird and Emery, 2009).

Many species of the corvid family hide food in stashes for later consumption. This caching behavior was investigated in a variety of experiments, that allowed to infer underlying cognitive abilities and demonstrated the importance of spatial information in their everyday life. Scrub jays, for example, were shown to memorize not only where they hid food, but also what and how long ago, suggesting an episodic-like memory (Clayton and Dickinson, 1998; Clayton et al., 2001). Given new information about a likely decay of one sort of cached food, they flexibly integrated that information and changed their behavior to only recover caches of a more durable sort of food even though it was less preferred (Clayton et al., 2003). Raby et al. (2007) showed that caching scrub jays also take into account knowledge of future events: the birds selectively cached food in a room where they learned they would be hungry the next day. While this study shows that corvids plan for the future, some corvid species can rely on an extensive memory of previous cache locations. In terms of long term recollection of cache sites, the Clark's nutcrackers stick out with their ability to remember their own cache locations close to a year after the caching event took place (Balda and Kamil, 1992). This species has not only an excellent spatial memory of own stashes but is also able to remember the cache locations of conspecifics that they observed during caching.

The ability to remember not only their own but also caches of conspecifics which were observed is called observational spatial memory. Observational spatial memory enables the pilfering of other birds' caches at a later time without the risk of being caught and is common in many corvid species. In term of their capacity for observational spatial memory, the Clark's nutcracker is surpassed by the Mexican jay. While Mexican jays remember cache locations of conspecifics after two days, the Clark's nutcracker is not. One explanation for this difference in observational spatial memory might be that the Clark's nutcracker lives in small groups or even solitary and the Mexican jay in large groups. Hence the Mexican jay is confronted with social challenges that could have led to the evolution of different cognitive abilities (Balda and Bednekoff, 1996; Bednekoff and Balda, 1996). In a similar line of argumentation, also the intelligence of humans might have evolved in response to living in large social groups (Dunbar, 1998). At least for corvids species living in large groups, the ability to not only remember their own, but also the cache sites of conspecifics led to an arms race in cache pilfering and protection strategies.

By investigating the various cache protection strategies, studies were able to unveil a part of the cognitive abilities that corvids are endowed with. To make it harder for pilfering conspecifics, ravens preferably cache out of sight or re-cache their food at a later time point, after being watched by a conspecific (Heinrich and Pepper, 1998; Bugnyar and Kotrschal, 2002). They also engage in cache protection in cases when they themselves cannot see but only hear a conspecific in a place from which the conspecific could observe them (Bugnyar et al., 2016). Emery and Clayton (2001) showed that also scrub jays re-cache food when they were observed during caching. Interestingly they only do so if they themselves have experience in pilfering others cache. This is argued to be an indication for projecting of own experience to conspecifics, suggesting theory-of-mind like abilities. Similarly, ravens took into account what knowledge another conspecific had when planning their own actions, potentially demonstrating a basic level of theory-of-mind (Bugnyar, 2011). A variety of other cache protection strategies, including caching selectively behind barriers out of sight or making fake caches to confuse observers (Heinrich and Pepper, 1998; Dally et al., 2005; Clary and Kelly, 2011; Legg and Clayton, 2014), were added to the repertoire of the corvid family and show their resourcefulness (Dally et al., 2006).The topic of cache protection strategies not only provides us with a window into the cognitive abilities of corvids, but it also shows that especially for corvids spatial information is a dominant feature affecting and determining their daily routine.

Previous work in our lab focused on carrion crows and investigated cognitive abilities in a highly controlled environment. The following studies show that the cognitive flexibility shown above also allows corvids to learn complex behavioral protocols. Wagener and Nieder (2017) and Veit et al. (2014) trained crows to remember the identity of a complex picture or a motion direction of a stimulus. Crows were also trained to the concept of greater than/less than (Moll and Nieder, 2014) and to discriminate the numerosity of dots from one to thirty

(Ditz and Nieder, 2015, 2016). Finally, carrion crows were also able to flexibly switch between a same and a different rule, that was instructed visually or auditory (Veit and Nieder, 2013). All these studies show that corvids and especially carrion crows can be trained to complex task protocols to investigate different cognitive abilities.

In summary, the reviewed studies demonstrate that corvids have impressive cognitive capacities and can be trained to abstract behavioral protocols. Although corvids are only distantly related to primates and mammals in general, they are equipped with a similar cognitive toolkit (Emery and Clayton, 2004; Clayton and Emery, 2015; Nieder, 2017b). Corvids are therefore a highly interesting model to study cognitive capabilities. The comparison to cognition in mammals is even more compelling, because both classes have drastically different brain architecture as will be shown in the next chapter.

1.3. Neuronal basis for cognitive abilities

The last common ancestor of birds and mammals lived around 300 million years ago (Jarvis et al., 2005). In the long time of parallel evolution, both groups evolved to species-rich classes with distinct differences in morphology. Beside obvious differences in body morphology, there are also differences in the brain architecture between both classes. This architectural difference can be seen in the telencephalon (endbrain). While the ventral parts of the telencephalon, containing the basal ganglia, are similar in birds and mammals, there are large differences in the dorsal part, that is called pallium. In mammals, neurons in the pallium are organized into multiple characteristic layers, and information is typically processed across these layers in a strict hierarchy. In contrast, the avian brain almost completely lacks layering and instead is organized into many clusters of neurons. Processing in the avian brain is carried out along connected clusters (Jarvis et al., 2005, 2013; Nieder, 2017a). Because the telencephalon is commonly thought to be the center of complex cognition, the different architecture in birds provides an interesting opportunity to compare the information processing on population and single cell level.

Despite architectural differences in organization of the avian and mammalian pallium, the flow of information follows a similar pattern in both endbrains. In the mammalian cortex information is processed in characteristic columnar microcircuits: Information input to layer 4 and output of information from layer 5, with hierarchical processing of information across the other layers between input and output. This processing of information across connected areas in a strict hierarchy is thought to underlie the computations that are needed for complex cognition (Calabrese and Woolley, 2015). Although this specific processing is not possible in a brain that lacks layers, studies suggest that a functionally similar pathway exists in the nuclear organized avian brain. Dugas-Ford et al. (2012) and Butler et al. (2011) described specific input cell-types in the avian pallium that receive the input to the pallium, and other cells-types that act as output units to other brain regions. These cell-types are proposed to be homologous to the mammalian cortex layers 4 (input) and 5 (output). Calabrese and Woolley (2015) investigated this processing circuit for auditory information and found functionally analogous avian processing steps also for the remaining mammalian cortex layers. These results suggest that a basic processing microcircuit was already present in a common ancestor (Dugas-Ford et al., 2012; Calabrese and Woolley, 2015).

Homologies between avian and mammalian pallial regions are still a subject of scientific debate. Different competing hypotheses propose homologies of the mammalian layered cortex to different regions of the avian brain. A recent hypothesis that combines previously stated hypotheses is the modified field hypothesis of homology. It proposes that the avian pallium as a field is homologous to the mammalian layered cortex and also to the nuclear-organized claustrum/amygdala complex (Jarvis et al., 2013). The homology of the avian and mammalian pallial regions also supports the above proposed shared way to process information, because it links the nuclear architecture of avian pallium to the layers of the mammalian cortex. Hence, the information processing in the pallium of birds and mammals seems to follow similar principles, which might be due to their common evolutionary origin.

1.3.1. Center for cognitive abilities in corvids

Cognitive functions, including the executive functions described in the previous chapters, are necessary for goal directed behavior. Previous studies in birds and especially corvids linked the *Nidopallium caudolaterale* (NCL) to complex cognitive functions. The NCL is an association area, located at the caudal end of the pallium. This area is reciprocally connected to secondary sensory areas of all modalities and also projects to motor output structures, the basal ganglia and limbic system (Leutgeb et al., 1996; Kröner and Güntürkün, 1999). In addition, NCL is characterized by strong dopaminergic innervation from the ventral tegmental area and substantia nigra (Wynne and Güntürkün, 1995; Durstewitz et al., 1999). This connectivity puts NCL in the perfect position to integrate sensory information with previous knowledge and influence behavior.

NCL maintains information in working memory. Diekamp et al. (2002a) trained pigeons on a delayed match to sample protocol, in which the animals had to remember a color over a variable delay period. After successfully training the animals, the NCL of one group was bilaterally ablated. After the surgery, behavioral performance of the group with NCL ablations was significantly lower compared to the control group. Interestingly, this impairment in performance was not due to problems in visual discrimination, as both groups performed equally in a respective test. These results indicate that NCL might be involved in maintenance of color information over a memory delay. Similarly, studies found an impairment in delayed alternation protocols after lesion of NCL (Mogensen and Divac, 1982, 1993; Gagliardo et al., 1996). These studies suggest that NCL is also participating in maintenance of spatial information, because the animals had to maintain the last chosen location in the delayed alternation tasks. One problem of lesion studies is that behavioral effects can also be explained by corollary effects, such as the severing of a pass-through projection between two other areas. It is therefore important to mention that Veit et al. (2014) demonstrated a direct involvement of NCL neurons in working memory. In this study crows were trained on a delayed match to sample task, were they had to maintain the identity of a picture over a delay period. Single neurons, recorded in NCL during that protocol, selectively encoded the picture identity both during visual presentation and over the subsequent delay period. This encoding of information during a delay period in the absence of visual stimulation is commonly interpreted as a hallmark of working memory.

NCL is suggested to be involved in motor processes. Different studies reported that inactivation of NCL also resulted in complete omission of behavior, indicating an involvement in motor processes (Lissek and Güntürkün, 2004, 2005; Lengersdorf et al., 2014a). Helduser and colleagues trained pigeons on a sequence of motor responses and subsequently inactivated NCL. This inactivation resulted in some animals in a complete suppression of the learned behavior, while in others the number of errors increased significantly compared to a control group. An analysis of the type of errors showed that NCL inactivation resulted in increased skipping of sequence steps or exhibiting of preservation (Helduser and Güntürkün, 2012; Helduser et al., 2013). This was interpreted as an indication that NCL is involved in the initiation of motor sequences. Kalt et al. (1999) trained head fixed pigeons on a go/no-go task, where the animals had to mandibulate in the response period after seeing one visual cue an withhold responses after seeing another. Single neurons in NCL selectively differentiated between go and no-go trials. This selective activity was interpreted as a motor plan in go trials but could also be explained by encoding of reward expectancy. Similar results in a go/no-go protocol were also reported by Starosta et al. (2013). The study of Lengersdorf et al. (2014a), that will be discussed in detail in the discussion, also reported NCL neurons that potentially encode a motor plan. But their results are ambiguous and could also be interpreted as an encoding of sensory information. Two studies investigated the involvement of NCL during movement execution (Lengersdorf et al., 2014a; Veit et al., 2015). In both studies, neurons encoded the direction of movement during movement execution in the response period. Summarized, studies hint towards an involvement of NCL in motor planning, while strong evidence places encoding of movement direction during execution in NCL.

NCL also represents higher cognitive functions. Veit and Nieder (2013) trained crows on a delayed rule-switching task. In the task animals had to remember a sample picture and after a delay either choose the same or a different picture. The same/different rule was instructed by visual or auditory rule cues between sample presentation and choice period. Single neurons in NCL differentiated between the rule concepts of same and different, demonstrating and involvement of NCL in abstract rule coding. This encoding of the rule was behaviorally relevant, because it was inverted in trials in which the wrong rule was applied. In addition to rule coding, NCL neurons encoded and maintained the numerosity of visual displays (Ditz and Nieder, 2015, 2016), and did so also without prior training of the animal (Wagener et al., 2018). Moreover, NCL was also shown to encode associations: Moll and Nieder (2015, 2017) trained crows on visual-auditory and auditory-visual match to sample protocols, where visual cues were associated with auditory cues and vice versa. After hearing or seeing a sample cue, the animal had to respond only to the associated cue of the other modality and had to withhold responses for all other cues. NCL neurons recorded during this task, displayed modality invariant responses for associated cue pairs. This representation of associated pairs was shown to emerge in NCL during learning of associations (Veit et al., 2015, 2017).

Above studies reveal the NCL as an association area involved in various cognitive functions in the avian brain. In the mammalian brain an association area with similar anatomical connectivity is the prefrontal cortex (PFC) (Miller, 2000;

Fuster, 2001, 2015; Miller and Cohen, 2001). Also PFC was previously shown to be involved in different cognitive functions like working memory (Niki and Watanabe, 1976; Funahashi et al., 1989, 1993b; Wilson et al., 1993; Rainer et al., 1998; Takeda and Funahashi, 2002), abstract rules (Wallis et al., 2001; Wallis and Miller, 2003; Bongard and Nieder, 2010; Eiselt and Nieder, 2013, 2014), and numerosity (Nieder et al., 2002, 2006; Nieder and Merten, 2007; Nieder, 2012; Viswanathan and Nieder, 2013, 2015). This similarity on both the level of connectivity and function resulted in the proposal of NCL as a functional analog of the mammalian PFC (Güntürkün, 2005; Nieder, 2017b).

2. Results

In the following chapters I will summarize the main results of the one publication and one manuscript included in this thesis. The full publications are attached as chapters at the end of the thesis (see Individual studies).

2.1. Chapter I: NCL neurons encode and maintain spatial information

The aim of this study was to investigate if neurons in the NCL of carrion crows encode the spatial location of objects in their visual field and if these or other neurons also maintain the spatial information over a delay period. A previous study by Veit et al. (2014) showed that NCL neurons encode the visual identity of objects and maintain this information via sustained activity over a delay period. Until now, the encoding of the spatial component of objects during sensory and working memory periods was not investigated. We therefore trained two carrion crows on a delayed response paradigm to test NCL neurons for an involvement in spatial information processing (Publication 1, Fig1A). Our delayed response task was adapted from an established protocol for investigating spatial working memory in primates (Funahashi et al., 1989). In our protocol, the crows were presented with a spatial cue that could appear at one of eight different spatial locations around a central fixation bar. The crows had to remember the spatial location of the cue over a delay period of variable length. At the end of the delay, they were instructed to select the memorized location by a peck on a touchscreen. Importantly, the cues only differed in its spatial location from trial to trial and were otherwise identical. Therefore, the information input to the animal from trial to trial differed only in the spatial location of the cue. In order to compare the neuronal activity during the main protocol with a situation with multiple cued locations, we introduced so called free-choice trials. In these trials two spatial locations were cued in parallel, and the animal was free to choose either of the locations in the later response period.

2.1.1. NCL neurons encode the spatial location of sensory input

After the animals successfully learned the protocol (Publication 1, Figure 1B), we recorded activity of 291 single neurons in the right NCL while the animals were engaged in the task (Publication 1, Figure 1C). We analyzed all neurons during the sensory presentation of the spatial cue to investigate if they preferred specific spatial locations. More than half of the recorded population of neurons

(55%; 161/291) were significantly modulated by spatial locations during cue presentation. These cue-selective neurons typically increased their firing rate when the cue was presented at a single or multiple neighboring spatial locations (example see Publication 1, Figure 2A). Over the whole population of 161 cue-selective neurons, the preferred spatial locations were distributed uniformly to all possible directions (Publication 1, Figure 2E). This cue-selective activity is most parsimoniously explained by a representation of current sensory input. To my knowledge this is the first report of encoding of sensory spatial information in the avian NCL.

2.1.2. NCL neurons maintain spatial information in working memory

To investigate the involvement of neurons in the maintenance of spatial information, we analyzed neuronal activity during the memory delay. Like in the sensory period, also in the delay neurons showed selective modulation for specific spatial cue locations. These neurons typically increased in firing rate for one or few cue locations in the absence of sensory stimulation (example see Publication 1, Figure 2B). More than forty percent (120/291) of all recorded neurons showed such delay-selective activity. Most of these delay-selective neurons (82/120) were not only maintaining stimulus location in the delay period, but already started to encode the location during stimulus presentation (Publication 1, Figure 2D). These neurons might be vital for the transition from the sensory to the memory period. Like the cue-selective population, also the population of delay-selective neurons preferred all possible directions equally (Publication 1, Figure 2F). This temporary maintenance of information via selective sustained activity in the absence of sensory stimulation is commonly interpreted as a correlate for working memory.

Because the cue location and the later response direction were identical, delay selective neurons could be interpreted as encoding the retrospective cue location or the prospective response location. A neuron that encodes a motor plan, should co-vary its firing rate with the animals' reaction time. This assumption was tested and validated previously in primates to distinguish working memory from response related neurons (Markowitz et al., 2015). In our data the large majority of delay-selective cells retrospectively maintained spatial information and only a minority encoded prospective response planning (9/112 neurons firing rate co-varied with the reaction time; Publication 1, Figure 3).

The spatial information represented in our population of recorded neurons is sufficient to decode the memorized location of each trial from population activity. We trained a k-nearest neighbor classifier to predict the cued location in a given trial based on the population activity of all recorded neurons. This classifier was able to successfully predict the cued location at any given time point during cue presentation and in the subsequent memory delay (Publication 1, Figure 4). This result shows that the spatial information inherent in the randomly sampled population of NCL neurons, would be sufficient to allow an ideal observer to solve the task in terms of memorizing the cue location.

NCL only maintains behaviorally relevant spatial information. During free-choice trials in which the animal could choose between two alternative spatial locations, only the later selected location was encoded (Publication 1, Figures 6 & 7). This indicates that NCLs role in maintaining of spatial information is not comparable to a simple sensory short-term buffer but to a selective representation of behaviorally relevant information.

2.1.3. Population code is stable within the delay

The population code that allows to read out the spatial cue location changes over time and differs between the sensory and memory period. We applied a cross-temporal classification analysis to tests if the population code that maintains the spatial information was stable or changed over time (Stokes et al., 2013). This classification analysis showed that the decoding schema, which allowed the read out of the spatial location during cue presentation, did not generalized to the later parts of the delay period and vice versa (Publication 1, Figure 5). At beginning of the delay, during the transition from a sensory to a memory representation, both codes were present in the population. These results show a stable population code during the delay period, that is different from the sensory cue period. This suggests one population code during the cue period representing sensory input, and another memory related code during the delay period.

2.1.4. Summary

This first study presents two important findings: first, NCL neurons encode spatial information during sensory stimulation. Second, NCL neurons also maintain the spatial information of an object over a memory delay, if this information is of behavioral relevance to the animal. The NCL as a population would be sufficient to read out the cued spatial location at any given moment during sensory stimulation or during the memory delay. This study suggests that the NCL is an integral system in spatial information processing for goal directed behavior, because it is involved in both sensory encoding and maintaining of behaviorally relevant spatial information.

2.2. Chapter II: NCL neurons encode planned movements and movement direction during movement execution

The previous study showed NCL's involvement in processing of sensory spatial information and the maintenance of that information in working memory. In this second study we wanted to investigate if the NCL also takes part in the planning of a behavioral response. Previous studies already presented NCL neurons that encode movement direction during movement execution (Lengersdorf et al., 2014b; Veit et al., 2015), while NCL's involvement in the planning of a motor response remained unknown. To clarify NCL's role in motor planning we trained two carrion crows on a spatial delayed response task that lacked the need for maintenance of information. The rationale behind the design of this task was that behaviorally relevant information would only be maintained in working memory, if that information was presented only for a limited time. Conversely, information presented continuously throughout a protocol, would not be maintained to save capacities for necessary processing. Thus, in contrast to the previous study, spatially selective information in this setting could not parsimoniously be explained by retrospective maintenance of information but more likely by the prospective encoding of a planned movement direction. This rationale was used previously in studies on primates to distinguish spatial working memory from motor planning activity (Funahashi et al., 1991, 1993a; Markowitz et al., 2015). In our spatial delayed response task, two of eight possible spatial locations were marked by grey boxes in each trial (Publication 2, Figure 1A & 1B). There was always a location on the left and the right side of the screen. A centrally presented rule cue instructed the animal to prepare a response to either the left or the right box location. Both the rule cue and the two potential target locations were presented continuously throughout the planning period, obviating the need for memorization of both locations and rule cue. The length of this planning period was randomly varied in length and followed by the response period in which the animal was instructed to rapidly perform a motor response (i.e. a peck) to the instructed location. Both the variable length and a short response window ensured that the animal prepared the motor response beforehand. In order to distinguish selective sensory activity to specific rule cues from movement preparation, each side was randomly cued by either a shape or a color rule cue (Publication 2, Figure 1B). In a subset of trials, we presented an ambiguous rule cue, that was inconclusive in its visual properties and accordingly in its rule meaning. In these trials, the animal could choose either of the presented locations during the response window. After the animals successfully learned the protocol, we recorded the activity of 408 single neurons in the left NCL of both animals.

2.2.1. NCL neurons encode a motor plan

More than one fourth of the recorded cells (29%; 117/408) selectively encoded the motor plan during the planning period. These neurons selectively preferred either trials with a specific target location (Publication 2, Figure 2B) or all target locations within a side (Publication 2, Figure 2A). This selective activity for a side cannot be explained by encoding of visual features of the rule cue, because the selective activity was not affected by the shape or color of the rule cue that instructed a side. Moreover, cells with a preference for a side could be interpreted as representing the abstract rule concept of 'choose left' or 'choose right'. It is however more parsimonious to assume a prospective encoding of the upcoming target location instead of the abstract concept of a rule. Consistent with this argument, neurons preferring single locations within a side cannot be explained by an abstract encoding of the rule and hence can only be interpreted to encode the motor plan. Over the population of neurons selective during the planning period, a large majority preferred planning of movements to the contralateral side (75% or 88/117 neurons preferring locations on the right side; Publication 2, Figure 2C). While this contralateral bias in the spatial representation is an interesting finding on its own, it is also another argument against an interpretation as an abstract rule signal because a single-sided preference for only one of two opposing abstract rules would be highly improbable. Thus, the selective activity of NCL neurons is most parsimoniously explained by encoding of a prospective motor plan.

The recorded population of NCL neurons was enough to allow a classifier to successfully predict the target location chosen in the response period, based on the activity during the planning period (Publication 2, Figure 5D). When the classifier predicted the wrong location, most erroneously predicted locations were within the correct side. This higher probability of wrong predictions within the correct side, can be explained by neurons that have a general preference for all targets within a side. A classifier was also able to predict the target location in ambiguous trials above chance (Publication 2, Figure 3). This correct prediction for trials without conclusive rule information, is another indication for an affiliation of the selective activity with the later behavioral response. These classification analyses show that the selective activity found in our recorded population of NCL neurons was sufficient to predict a future motor response.

2.2.2. NCL neurons encode movement direction during execution

Nearly forty percent of all recorded cells (39%; 158/408) encoded the direction of movement during movement execution. A typical neuron increased its firing rate selectively for a single or multiple neighboring target locations while the bird was performing the movement towards the targets (Publication 2, Figure 4A). In contrast to the planning period, the preferred directions in the population of neurons selective during movement execution were evenly distributed to all possible directions (Publication 2, Figure 4B). When training a classifier to predict the movement direction of the animal based on neuronal activity during the movement, the classifier was not only able to successfully predict the target location above chance but with a noteworthy high accuracy of above 70% (Publication 2, Figure 5E). In addition to this remarkable decoding performance, and in contrast to the classification during motor planning, most erroneous classifications were to spatially adjacent locations. These results hint toward a more precise encoding of spatial information during movement execution compared to the planning of the movements. A more precise spatial tuning during movement execution compared to planning was also found, when comparing the tuning broadness of selective cells in the planning and response period (Publication 2, Figure 5B & 5C). While the encoding of spatial movement direction during execution was already reported in two previous studies (Lengersdorf et al., 2014a; Veit et al., 2015), our presented data extends the current knowledge by showing NCL neuron tuning to eight individual spatial locations.

2.2.3. Summary

The data presented in this study contains two main findings on NCL: first, NCL is involved in planning of motor responses. This is shown on the level of single neurons that encode the direction of a future movement and on population level. Second, NCL also represents the current movement direction during the execution of a movement. Both findings suggest a close connection of NCL to brain areas involved in the execution of motor responses.

3. Discussion

Goal directed behavior governs an important part of our daily life. As I stated in the introduction, goal directed behavior in its simplified form can be viewed as perception-action cycles. At beginning of a cycle, sensory information needs to be filtered, maintained, and evaluated for possible behavioral responses. In a second step, a response that is believed to have a goal directed effect must be selected and prepared. Subsequently, the action must be executed at the appropriate time, completing the perception-action cycle. In my PhD thesis, I show involvement of the avian association area *Nidopallium caudolaterale* (NCL) in all aforementioned steps of information processing for goal directed behavior, emphasizing the central role of NCL in cognitive processes in the avian brain.

3.1. Comparison to previous findings in avian NCL

Previous studies demonstrated that neurons in NCL encode complex cognitive task parameters like abstract rules or inter- and cross-modal associations (Veit and Nieder, 2013; Moll and Nieder, 2015, 2017; Veit et al., 2015, 2017). While NCL neurons were also shown to encode and maintain the stimulus identity of visual items (Veit et al., 2014), this was not shown for the spatial location of objects. In this chapter I will review the previous knowledge about single neuron activity in NCL and set it into context to the findings in my thesis.

3.1.1. Encoding of visual sensory input in NCL

In this thesis I demonstrate that NCL neurons encode the spatial location of visual stimuli. Earlier studies in the NCL of crows and pigeons showed that discrete populations of NCL neurons represent different features of visual stimuli during sensory stimulation. These neurons represented the color, identity, or numerosity of presented visual stimuli (Veit et al., 2014; Ditz and Nieder, 2015; Johnston et al., 2017; Wagener and Nieder, 2017; Wagener et al., 2018). In combination with my thesis, these findings suggest that NCL neurons are encoding different visual features of stimuli when they are of behavioral relevance. My findings extend the current knowledge about the encoding of sensory information in NCL and might enable new experimental designs that rely on encoding of spatial information.

3.1.2. Involvement of NCL in working memory

My thesis is the first study to show that NCL maintains the spatial location of visual objects over delay periods. Previous studies already showed that NCL participates in working memory, with single neurons in NCL maintaining information in the absence of sensory stimulation. In the study by Veit et al. (2014), NCL neurons maintained the identity of pictures over a delay period, allowing crows to solve a delayed match to sample protocol. In a similar protocol, Ditz and Nieder (2016) showed that crow NCL neurons also maintained the numerosity of items. Also in the pigeon NCL neurons maintained information over delay periods (Diekamp et al., 2002b; Johnston et al., 2017). An important addition from the data presented in this thesis is, that spatial information is only maintained when it is of relevance for later behavior. This suggests the NCL as a flexible storage, that can maintain a variety of different visual features when they are of behavioral relevance.

3.1.3. NCL and motor planning

The data presented in this thesis confirms prior assumptions and places NCL in the process of motor planning. Planning of a motor response was previously investigated only in few studies in pigeons.Kalt et al. (1999) and Starosta et al. (2013) trained pigeons on go/no-go protocols and found NCL neurons that differentiated between trials with a later motor response and trials without a response. While this activity could be interpreted as a neuronal signal prospectively encoding the planned motor response, it can also be explained by encoding of an expected reward in go trials against no reward in no-go trials. Another study tried to solve this ambiguity by training pigeons to respond to a target on either the left or right side, depending on a previous instructional cue (Lengersdorf et al., 2014a). NCL neurons in Lengersdorf et al. (2014a) differentiated between responses to the different sides in a task period before the response was executed. However, the authors instructed the two sides with a stimulus that differed only in luminance, with brighter intensities instructing the left and darker intensities instructing the right target location. Thus, the potential selectivity for a later response location can also be explained by a simple encoding of stimulus luminance. In the behavioral protocol used in my thesis, we instructed each target side with two different rule cues that differed in shape and color. These different rule cues allowed us to distinguish sensory activity encoding visual properties of a rule cue from activity encoding instructed target location. Therefore, the encoding of individual future target locations presented here is not confounded by the sensory properties of the rule cue. Together with the participation in spatial working memory shown above, my results suggest NCL as a potentially vital actor in the creation of a motor plan based on available information.

3.1.4. NCL activity during movement execution

My study also shows that NCL neurons encode the movement direction at the time of movement execution. This finding corresponds to previous findings in the aforementioned study by Lengersdorf et al. (2014a) or in the study by Veit et al. (2015). In both studies NCL neurons differentiated the direction of movement while the animals were performing the movement. In the Lengersdorf et al. (2014a) study, neurons differentiated between left and right. Veit et al. (2015) investigated neuronal activity during the test period of a delayed match to sample protocol with four different test images. NCL neurons showed increased activity during movement towards specific test image locations irrespective of the image presented at that location. My findings confirm this encoding of movement directions in NCL by presenting neurons that differentiate between movements to eight different target locations. This indicates that the selectivity during movement execution is not a side effect created by a specific behavioral protocol.

3.2. Comparison to previous findings in mammalian PFC

Previous studies suggested the mammalian PFC as a functional analog to the avian NCL, based on anatomical connectivity and functionality (Güntürkün, 2005; Nieder, 2017b). In contrast to the few studies that looked at processing of spatial information in the avian NCL, a large number of previous studies investigated the processing of spatial information in the mammalian PFC. In this chapter, I will review previous studies in PFC and compare them to the findings of this thesis. Following the structure of the previous chapter, I will first review studies that show encoding of spatial information during sensory stimulation, followed by studies on spatial working memory, motor planning, and finally representation of movement direction during movement execution.

3.2.1. PFC encodes the spatial location of sensory input

Like the avian NCL, also the mammalian PFC encodes the spatial information of visual stimuli. The mammalian PFC is reciprocally connected to visual sensory areas (Miller, 2000; Fuster, 2001; Miller and Cohen, 2001). Because of the input from these areas, it is not surprising that PFC neurons also encode the spatial location of visually presented objects. In a classic study by Niki (1974), monkeys were trained on a manual delayed response task with a button on the left and another on the right side. In the cue period one button was illuminated, indicating that it should be pressed in the later response period. During illumination neurons in PFC differentiated between the two possible button locations. This sensory encoding of spatial locations was also shown in a oculomotor delayed response protocol with eight possible target locations (Funahashi et al., 1990). Interestingly, the latter study found that the large majority of PFC neurons preferred spatial locations in the visual field contralateral to the recorded hemisphere. When compared to my findings on NCL neurons, we find a highly similar encoding of spatial information in single neurons of both areas. However, when looking at the population level we did not find the bias for contralateral spatial locations in NCL. This suggests that while the general coding principle for sensory spatial information is the same in both areas, the input of sensory information to both areas differs.

The difference in the population preferences could be explained by differences in the visual pathways between birds and mammals. In both birds and mammals two visual pathways from the retina to higher brain areas exist. The geniculo-striate pathway is the major visual pathway in primates, while in birds the tectofugal pathway is dominant. These pathways are not homolog, so the major visual input pathways differ between both classes (Bischof and Watanabe, 1997; Güntürkün, 1997, 2000; Shimizu et al., 2010). Due to the semi-decussation of the optic nerve in primates, the geniculo-striate pathway mainly contains visual information from the contralateral visual field. The separation of visual hemifields is maintained until the end of the path in visual cortex. This dominance of contralateral input to the early visual cortex is a likely explanation for the contralateral preference of PFC neurons. In contrast, the tectofugal pathway in birds performs a complete decussation and results in a monocular representation of visual information in the optic tectum (Cowan et al., 1961). However, already on the way to the next area along the pathway, information is projected both contra- and ipsilateral (Saleh and Ehrlich, 1984; Bischof and Niemann, 1990). This results in an earlier representation of both ipsi- and contralateral information in the dominant visual pathway of birds compared to mammals. Early bilateral representation of information might be the reason for the equal representation of both hemifields in birds.

3.2.2. PFC participates in spatial working memory and motor planning

In coherence with my data on NCL, also PFC was previously shown to be involved in both retrospective maintenance of spatial information and prospective encoding of response locations. The maintenance of spatial information was shown among others in the classic study by Funahashi et al. (1989). In their study monkeys were trained on an oculomotor-version of the delay response paradigm. The spatial location of a cue had to be maintained over a memory delay, after which a saccade to the cued location had to be performed. Single neurons in PFC showed selective activity to individual cue locations and maintained this activity over the delay period. These delay selective neurons were proposed to have so-called memory fields, in analogy with the receptive fields of sensory neurons. The maintenance of spatial information by selective delay activity was also shown in other studies (for example Rainer et al., 1998; Murray et al., 2017). Sawaguchi and Iba (2001) were able to show that when they temporally inactivated small portions of PFC, monkeys were shown to have deficits in remembering spatial locations. These deficits were not explained by motor problems but could be due to inactivation of neurons with corresponding memory fields.

Apart from the retrospective maintenance of cue locations, studies were also able to show that neurons encoded the prospective target locations, indicating an involvement in motor planning. Hasegawa et al. (1998) trained monkeys to associate central color cues with response directions to the left or right. They found that PFC neurons encoded the response direction in the delay period before the motor response. Similarly, Quintana and Fuster (1999) and Wallis and Miller (2003) reported neurons that encoded the target location of an impending response.

While all above studies investigated involvement of PFC in either working memory or motor planning, several studies tried to distinguish the involvement of PFC in both processes in a single study. Funahashi et al. (1993a) extended the previously explained oculomotor delayed response task with an additional condition in which cue location and response location were opposite. This allowed to differentiate neurons that encoded the spatial location of the cue (same response in both conditions) from neurons that encoded the future response direction (differential response in the two conditions). Both neurons encoding retrospective and prospective spatial information were found, with most selective neurons encoding the retrospective working memory information. Similar results were also shown in other oculomotor delayed response studies (Takeda and Funahashi, 2002) and for manual responses (Niki and Watanabe, 1976). Markowitz et al. (2015) used a different approach that is similar to the line of argumentation in my thesis. They distinguished maintenance in working memory from motor planning activity by recording neurons during memory guided and visually guided saccades. Their results suggest encoding of both maintenance and motor planning information in anatomically distinct PFC populations. In addition, Markowitz et al. (2015)) showed that only neurons that encode a prospective motor plan have firing rates that are correlated with the animals reaction time.

In a follow up analysis on the same dataset presented by Funahashi et al. (1989), the same group showed that the memory field of delay selective neurons were to a majority in the contralateral visual field (Funahashi et al., 1990). This higher frequency of memory fields in the contralateral visual field could be explained analogous to the higher frequency of contralateral receptive fields during sensory stimulation described above: The dominant visual pathway in mammals' projects only contralateral visual information to early visual areas in the cortex. This biased input in early visual areas could be carried through various processing steps to the PFC.

None of the above studies analyzed the population of motor planning neurons for a preference for planning movements to the contra- or ipsilateral side. Data presented by Asaad et al. (2000) indicates that PFC encodes motor plans to the ipsi- and contralateral side with equal frequency. They recorded PFC neurons while the monkeys were engaged in a spatial delayed response task and an association task. In the spatial delayed response protocol, a spatial location on the left or right was cued with a spatial cue, and after a memory delay the cued spatial location had to be selected with a saccade. In the association protocol, the target location was not cued by a spatial cue but with a centrally presented picture that was associated with either the left or the right side. During the spatial delayed response protocol, a majority of PFC neurons encoded target locations on the contralateral hemifield. However, in this protocol the cue and target location were identical so the selective activity could be interpreted as both a prospective motor plan for the target location and a retrospective memory of the cue location. In the association task, the cue location and target location were distinct. Hence, spatially selective activity could not be interpreted as a retrospective memory of the cue location but rather as a prospective motor plan. In this association task, a similar number of neurons preferred motor plans to the contra- and ipsilateral side, suggesting a balanced frequency of side preferences in motor planning neurons.

Summarizing, different experimental approaches demonstrated coherently that neurons in PFC both retrospectively maintain spatial information and prospectively encode the target location of an impending response. These findings in the mammalian PFC parallel the results of my thesis in the avian NCL: first, both PFC and NCL single neurons maintain spatial information over memory delay period. Secondly, both areas contain single neurons that are involved in motor planning by representing the spatial location of a future response target. Comparing the populations of spatial working memory neurons, neurons in the PFC preferentially maintain information from the contralateral visual field. This contralateral preference is not found in spatial working memory neurons in NCL. Conversely, the population of motor planning neurons in PFC were evenly encoding movements to both hemifields, but motor planning neurons in NCL preferentially planned movements to the contralateral. This similarity in processing of information on single cell level combined with the difference in information encoded on population level suggests networks that process information similarly but differ in their input. The difference in information maintained, could arise from differences in visual pathways described above.

3.2.3. PFC activity during movement execution

Another parallel between NCL and PFC is found in the encoding of movement direction during movement execution. Funahashi et al. (1991) found single neurons that selectively preferred specific saccade directions during the response period, when analyzing the oculomotor delay response dataset mentioned earlier. Because they were constantly tracking the eye movement of the animals, movement direction selective neurons could be separated in two groups: one group with selective activity before saccade onset and another that exhibited selective activity after the saccade. Pre-saccadic neurons slightly preferred movement to the contralateral visual field on population level, while post-saccadic neurons were equally distributed with their preference for both visual hemifields. Similar results were also found in other studies using oculomotor delayed response protocols (Hasegawa et al., 1998; Takeda and Funahashi, 2002) and for hand movements (Niki, 1974). One might wonder why an area like the PFC, that encodes highly abstract information like for example rules (Wallis et al., 2001), is encoding non-cognitive motor directions. Takeda and Funahashi (2002) suggest that this selective activity could be a feedback signal from motor areas that could be used to terminate the maintenance spatial information in working memory. The interpretation as a feedback signal could also explain the encoding of movement directions in the avian NCL. This interpretation would indicate that NCL is not directly involved in the execution of a motor response but receives motor information to be able to adjust planned responses and working memory content.

3.3. Conclusion

This thesis presents data that shows that the avian NCL is involved in all processing steps necessary for goal directed behavior. NCL neurons encode spatial information during visual presentation and maintain this information in working memory if behaviorally relevant. At the same time, NCL also encodes the location of a planned motor response. Finally, NCL neurons encode the direction of movement during execution of goal directed behavior. These results paint the picture of NCL as a system that integrates sensory information and utilizes this information for planning of behavioral response. My findings parallel the results of previous studies on spatial information representation in the mammalian PFC. Hence, my thesis adds another functional similarity between these anatomically different brain areas in species separated by more than 300 million years of parallel evolution.

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Part II.
Individual studies

Statement of contributions

This thesis comprises one publication and one manuscript in preparation for submission.

1. **Rinnert P**, Kirschhock ME, Nieder A (2019) Neuronal Correlates of Spatial Working Memory in the Endbrain of Crows. *Current Biology* 29:2616-2624.

   I designed the Task with A. Nieder. I trained the crows and performed electrophysiological recordings with M. E. Kirschhock. I analyzed the data. I wrote the manuscript with A. Nieder. The full publication is found in the chapter Publication 1.

2. **Rinnert P**, Nieder A (manuscript) Single neurons encoding spatial motor planning and execution in crow endbrain.

   I designed the Task with A. Nieder. I trained the crows and performed all electrophysiological recordings. I analyzed the data and wrote the manuscript. The full publication is found in the chapter Publication 2.

Publication 1: Neuronal Correlates of Spatial Working Memory in the Endbrain of Crows

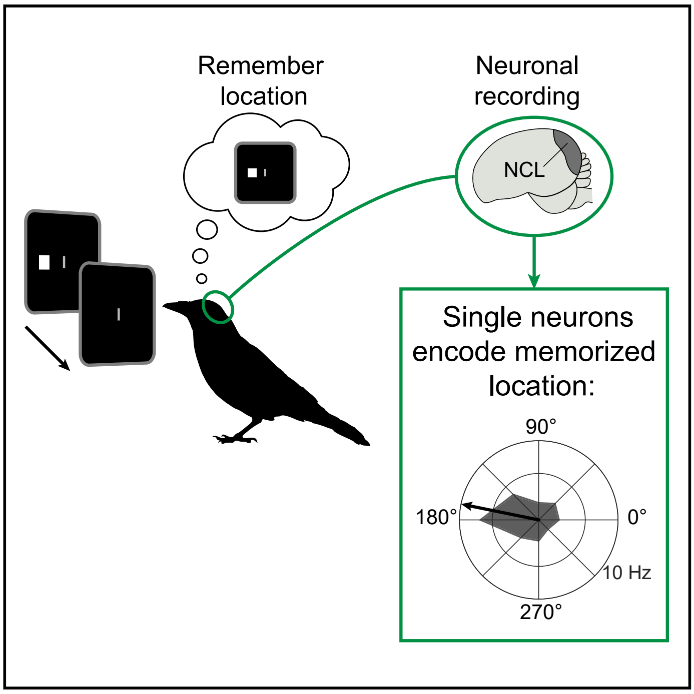
Rinnert P, Kirschhock ME, Nieder A (2019) Neuronal Correlates of Spatial Working Memory in the Endbrain of Crows. *Current Biology* 29:2616-2624.

Current Biology

Article

Neuronal Correlates of Spatial Working Memory in the Endbrain of Crows

Graphical Abstract



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In Brief

In crows trained to memorize the variable location of a visual item, Rinnert et al. show that neurons in the endbrain area *Nidopallium caudolaterale* are tuned in a behaviorally relevant way to individual preferred locations during working memory and are reminiscent of the convergently evolved primate prefrontal cortex.

Highlights

- Crows were trained to flexibly remember the variable location of a visual item
- NCL neurons were selectively tuned to spatial location in working memory
- Neurons stably maintained spatial information throughout the working memory period
- Spatially tuned neurons predicted the crows' future choices

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Neuronal Correlates of Spatial Working Memory in the Endbrain of Crows

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SUMMARY

Birds are renowned for their excellent spatial cognition. Corvid songbirds, in particular, rely on explicit representation of spatial cues in memory when caching food and retrieving caches for later consumption. However, the neuronal correlates of flexible spatial memory abilities are largely unknown in birds. We therefore trained carrion crows (*Corvus corone*) on a spatial delayed-response task in which they had to maintain the variable location of a visual item for a few seconds in working memory. After the crows performed this task with high precision, we recorded single-cell activity from the associative endbrain area *Nidopallium caudolaterale* (NCL) in the behaving crows. A large fraction of NCL neurons were tuned to individual preferred locations and selectively maintained the spatial location of items in working memory. A comparison of firing rates with reaction times suggested that the majority of delay-selective neurons represented stored location information rather than motor preparation. Almost 30% of all recorded neurons were tuned during both visual presentation and memory delay, and their spatial tuning was significantly correlated. The population of recorded neurons stably maintained spatial information over the course of the working memory period. Importantly, the neural responses of spatially tuned neurons were relevant for the crows' choices and allowed a statistical classifier to predict the subsequently chosen target location in free-choice trials. Our findings demonstrate the pivotal role of the avian NCL in spatial working memory that is reminiscent of the function of the convergently evolved primate prefrontal cortex in spatial working memory.

INTRODUCTION

Birds possess excellent spatial memory [1, 2]. Navigating birds, such as homing pigeons or migratory birds, travel hundreds of kilometers to reach precise target locations. Food-storing birds face similar challenges of spatial cognition when they create food caches during times of resource abundance for later retrieval during times of scarcity. Many corvid songbirds (jays and crows) cache food for later consumption and rely on precise spatial memory to retrieve their caches. In addition to their own caches, some corvid species also remember and later pilfer the caches of conspecifics [3, 4]. This led to the development of sophisticated cache protection strategies that again rely on flexible spatial cognition [5]. Corvids are also known to flexibly update their spatial memory: they switch from recovering perishable to non-perishable food after longer delays between caching and recovery [6], thus integrating information about the decay progress of caches [7]. To succeed in these situations, birds rely on the explicit representation, memorization, and manipulation of visual spatial cues in working memory, their visuo-spatial sketchpad. While the working memory capacity of pigeons and crows for object identity [8–10] and object categories [11–13] has been explored in some detail, their capacity to memorize the location of objects is largely unknown. Despite the importance of spatial working memory for birds, and corvids in particular, the neuronal correlates of this important type of working memory remain unknown.

We therefore explored the single-neuron mechanisms of visuo-spatial working memory in the telencephalic area known as *Nidopallium caudolaterale* (NCL) of behaving crows. The avian NCL is a high-level association area that receives input from all sensory modalities, interacts with long-term memory-related structures, and projects to premotor brain areas [14, 15]. As reflected by its anatomical connections, the corvid NCL plays an important role in a variety of cognitive functions [16–18]. Despite the independent and anatomically distinct evolution of avian and mammalian endbrains, the NCL is therefore considered to be the functional equivalent of the prefrontal cortex (PFC) [14, 15, 19], which enables working memory and cognitive control in primates [20–24]. Single-cell recordings showed that the NCL is involved in short-term memory representations: NCL neurons show selective delay activity in response to the identity of specific visual images and auditory events that serves to bridge temporal gaps in delayed-response tasks [25–27]. However, in ecologically relevant situations, such as the foraging situations described above, object location is another important feature that needs to be kept in mind in order to successfully solve tasks. To explore the neuronal mechanisms that allow birds to remember object locations, we recorded single-cell activity in crows performing a task that required visuo-spatial working memory.

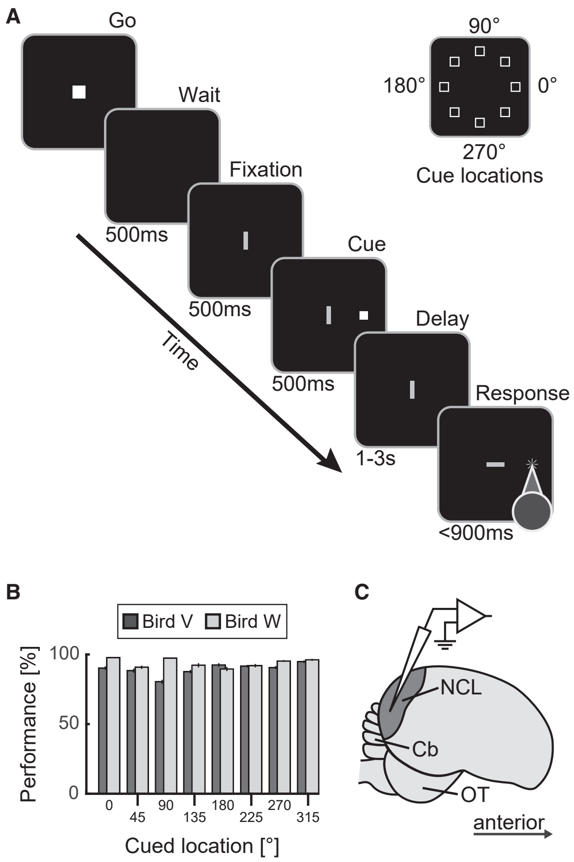

Figure 1. Spatial Delayed-Response Task and Behavioral Performance

(A) Behavioral task. The crow initiated a trial by positioning its head in front of the display in the go period. The go period was followed by a black screen (wait period, 500 ms). Next, a small vertical line appeared as fixation stimulus (fixation, 500 ms), which the crow was trained to observe and only respond when it changed its orientation. During the cue period, a peripheral cue (white square) was presented for 500 ms in one of eight possible locations (see inset "cue locations"). The bird had to memorize the location of the cue over the following variable delay period (duration between 1 and 3 s). Once the fixation line changed its orientation, the crow was required to respond as quickly as possible (within 900 ms) by pecking at the remembered location.

(B) Average behavioral performance for the eight cue locations over all recording sessions for bird V (black bars) and bird W (gray bars). Error bars indicate the standard error of the mean (SEM).

(C) Lateral view of a crow brain with the Nidopallium caudolaterale (NCL, shaded) located inside the telencephalon. Cb, cerebellum; OT, optic tectum.

RESULTS

We trained two hand-raised carrion crows [28] on a spatial delayed-response task in which they had to memorize for a few seconds the variable location of visual items displayed on a touchscreen (Figure 1A). In every trial, one of eight circularly arranged spatial locations was briefly cued by a gray square. After the cue had disappeared, the crows were required to remember the spatial location for an unpredictable time delay ranging from 1 to 3 s. After this delay, they were instructed to peck at this location on the all-black screen. To ensure that the crows maintained a stable visual field throughout the trial, they were additionally trained to fixate a central fixation target. The change of the fixation target after the variable delay of 1 to 3 s instructed the crows to peck at the previously cued location within 900 ms. Responses later than 900 ms were not rewarded. This forced the crow to look at the center of the screen throughout the trial in order not to miss the changing of the fixation target. Both birds performed above 80% for all eight cued locations over all recording days (Figure 1B).

Single Neurons Encode Cued Location in the Cue and Delay Period

While the crows performed this task, we recorded the activity of 291 single neurons in the right NCL [29] (Figure 1C). The cued location selectively modulated the activity of single neurons during the cue and delay period. An example neuron is shown in Figure 2A. This cell selectively increases its firing rate after onset of the bottom-left cue (location 225°) and the cues adjacent to this preferred location. After the cue vanishes, the firing rate returns to baseline for all locations. For each of the eight locations, an individual vector with a length corresponding to the average firing rate to a given location was calculated. The preferred direction of a neuron was defined as the angle of the average vector resulting from vector addition of all eight vectors [30] (Figure 2A, right panel). Neurons like this encode the location of the cue during its visual presentation and will be called cue-selective neurons. Other neurons modulated their firing rates only while the crows memorized the cued location in the delay period. Figure 2B shows such a delay-selective neuron that signals the right location (0°) only during the delay when the visual information had already disappeared. A third group of neurons, finally, responded selectively both during the cue and the subsequent delay period. The neuron in Figure 2C increases its firing rate for cues at locations 225°–315° (bottom locations) already during the end of the cue period. The selective responses are then maintained over the entire delay period. Such cue-and-delay-selective neurons bridge the temporal gap between cue presentation and the required pecking response.

A Large Fraction of NCL Neurons Encodes Spatial Information

We used analyses of variance (one-factorial ANOVA; $p < 0.01$) to test if the activity of a single neuron encoded the cued location. During the cue period, 55% (161/291) of all neurons were significantly tuned to the cued location (Figure 2D, example in Figure 2A). The preferred directions of these spatially tuned cue-selective neurons, as defined by the averaged firing rate over all locations, were equally distributed across all possible locations (Figure 2E). An equal number of those neurons preferred stimuli on the left and right side of the screen, respectively (55% versus 45%; binomial test, $p > 0.05$).

During the delay period, 41% (120/291) of all neurons were significantly encoding the memorized cue location (Figure 2D, examples in Figures 2B and 2C). The preferred directions of delay-selective neurons were distributed about equally to all

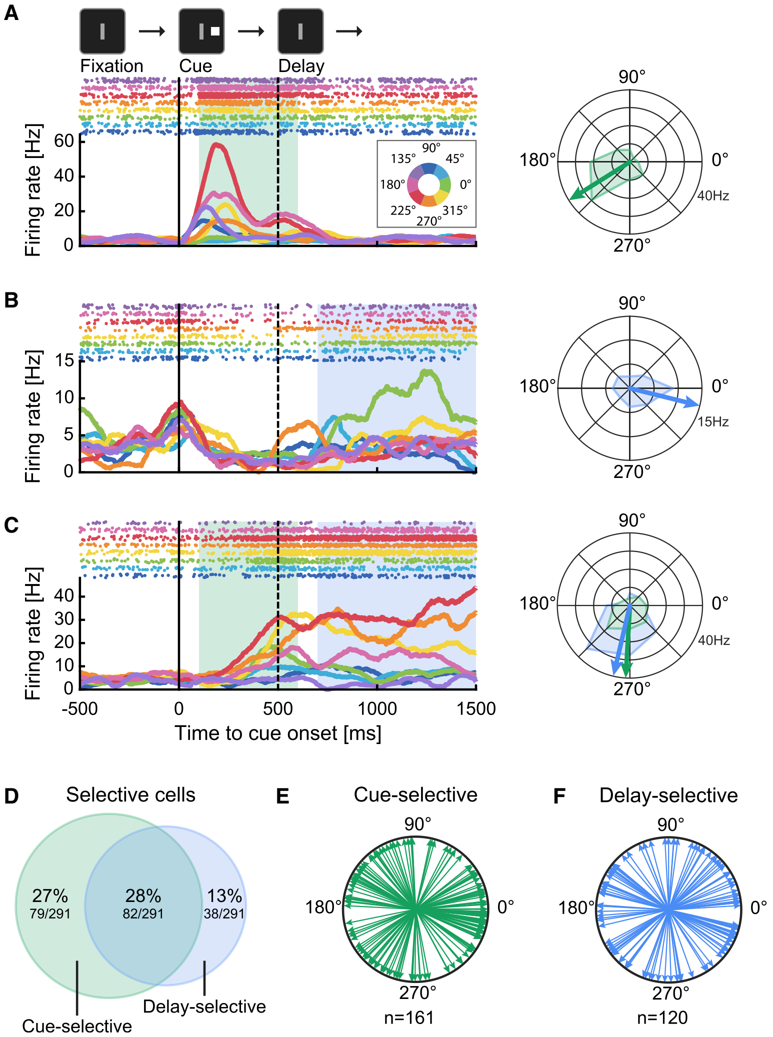

Figure 2. Single Neuron Responses to Spatial Locations

(A–C) Example neurons with cue-selective (A), delay-selective (B), and cue-and-delay-selective (C) activity. The left side shows dot-raster histograms (top) with corresponding spike-density functions (bottom). In dot-raster histograms, each dot represents an action potential, and each line represents a trial. Trials are sorted and color coded according to the cued location (see inset in A). Spike-density functions show the smoothed average firing rate over all trials for a cued location. Solid vertical lines represent the beginning of the cue period. Dashed vertical lines represent the beginning of the delay period. The right side shows polar plots with the firing rate for each cued location. The filled area in polar plots represents the average firing rate for the respective time window shaded in color in the respective spike-density histograms on the left side (green for cue period, blue for delay period). The colored arrow represents the preferred direction of a neuron.

(A) Example neuron tuned to location 225° (bottom-left) during the cue period.

(B) Example neuron tuned to location 0° (right) during the delay period.

(C) Example neuron tuned to cue location 270° (bottom) during late cue and the entire delay period.

(D) Venn diagram showing percentage of cue-selective neurons (green), delay-selective neurons (blue), and cue-and-delay-selective neurons (overlap).

(E and F) Preferred direction of all cue- and delay-selective neurons.

(E) Polar plot with arrows representing the preferred direction of each cue-selective neuron (n = 161).

(F) Same as in (E), but with preferred directions of all delay-selective neurons (n = 120).

possible locations (Figure 2F). Also, the proportion of delay-selective neurons preferring the left (47%) or right side (53%) of the screen was about equal (binomial test, $p > 0.05$).

Most of the location-selective neurons, i.e., 28% (82/291) of the whole population of neurons, were significantly tuned in both the cue and delay periods (Figure 2D). We calculated a circular-circular correlation between the preferred direction in the cue and delay periods. The preferred direction of the cue and delay period in individual neurons was significantly correlated (circular-circular correlation, $\rho_{cc} = 0.52$, $p < 0.01$).

The Majority of Delay-Selective Neurons Are Storage Rather Than Response Units

The spatial delayed-response task requires the crows to store location information in working memory but may also allow them to prepare a response during the delay period. Our task shares this problem of a potential combination of working memory and response preparation in the delay period with the classical oculomotor delayed-response task (ODR task) used for decades to study spatial working memory in monkeys [31]. We therefore investigated whether and to what extent persistent activity in NCL is composed of separable memory storage and response preparation activities. We reasoned that the memory storage and response preparation activities toward the end of the delay should differ in their relationship to the crows' pecking reaction time (RT). More precisely, we expected the discharges of neurons that encode motor preparation responses to co-vary with future RT. In contrast, this effect is not expected in memory storage units because only response preparation processes influence the timing of behavior after the delay. This rationale had previously been applied to differentiate storage and response modes in single PFC neurons of monkeys [32].

We therefore analyzed the population of delay-selective neurons for an increase in firing rate toward the end of the delay period (last 500 ms) as a function of RT. For any given neuron, we compared the firing rates occurring in the 50% fastest trials with those elicited in the 50% slowest trials. A total of 112 cells

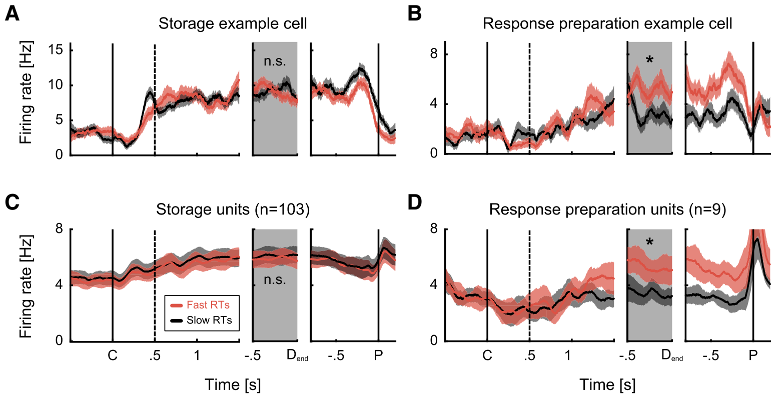

Figure 3. Delay-Selective Neurons Can Be Divided in Storage and Response Units

(A and B) Examples for a storage neuron (A) and response neuron (B). Spike-density histogram of neuronal activity is averaged for trials with fast reaction time (red) and slow reaction time (black). The left panel presents data aligned to the cue onset, showing activity during fixation, cue, and first second of the delay period. The middle panel presents data aligned to end of the delay period (D_{end}). The right panel presents data aligned to the peck on the screen (P). The gray area marks the analysis window.

(A) The firing rate of a storage neuron at the end of the delay is not affected by reaction time.

(B) A response-preparation neuron shows significantly increased activity for trials with fast reaction time.

(C and D) Activity of the population of storage units (C) and response preparation units (D). The shaded area in the graphs represents the standard error of the mean (SEM). The Asterix marks a significant difference in firing rates ($p < 0.05$) between fast and slow reaction-time trials.

(of the original 120 delay-selective cells) with at least 20 trials for both fast and slow RTs were included. Only firing rates to the preferred and the two neighboring locations were analyzed. We found that only 8% (9/112) of delay-selective neurons showed an increased firing rate for trials with a fast RT (Mann-Whitney-U test; $p < 0.05$) (Figure 3). Thus, only 8% of the delay-selective neurons were identified as “response-preparation” units, whereas 92% are considered as “storage” units.

Population Activity Encodes Cued Location

The activity of the entire population of recorded neurons, irrespective of their tuning behavior, encoded the cued location throughout cue and delay periods. To explore if an ideal observer could predict the cued location throughout the trial based on the activity of the neurons, we used a k-nearest neighbor classifier. We created a pseudo-population from all recorded neurons that were recorded for at least 20 correct trials for each location and performed a 5-fold-crossvalidation analysis in a sliding window over the trial ($n = 186$, $k = 5$, 100 ms window size, 20 ms steps). To compare the resulting decoding performance to chance level, we subsequently permuted the labels of the classifier 50 times and performed the same 5-fold-crossvalidation. The whole procedure was repeated 20 times to account for differences in trial selection. Decoding performance was defined as the average decoding performance across the true label cross-validations (20 repetitions). The decoding performance was defined as being significantly above chance if the decoding performance was above the 95^{th} percentile of the permuted data (1,000 or 50 × 20 repetitions). As expected, the classifier performance was at chance level (12.5% for eight locations) during the fixation period (Figure 4). However, shortly after the cue was presented, the decoding performance increased significantly above chance level and peaked around 35% accuracy. For the time of cue presentation and 200 ms into the delay, the performance remained at a similar level around 30%. Over the rest of the delay period, decoding performance slowly decreased to around 22% but stayed significantly above chance level. Around the time of movement execution—and shortly before the peck on the screen was registered—decoding performance increased again to a level around 35%. This analysis shows that the neuronal population is able to continuously maintain the spatial information based on overall firing rates throughout the course of a trial from cue presentation and delay period until a movement had to be executed.

Stable Population Code across the Delay Period

Population codes may change from stimulus presentation to working memory periods, and they may even change dynamically within the ongoing delay period [33, 34]. We therefore explored the consistency versus dynamics of population coding across the different task period and within the delay period. To that aim, we segregated the task phase into six 500-ms time windows (fixation, sample, first, second, and last 500 ms of delay windows, and respond window), trained a k-nearest neighbor classifier in each specific time window, and then tested the classifier’s performance in the different time windows of the task. A high classifier performance would suggest coding consistency across the different periods of time during the task, whereas low performance would suggest a change in population code from one time segment to the next. As expected from the previous analysis (Figure 4), decoding performance was significantly above chance whenever the classifier was trained and tested within the same time window (gray bars in Figures 5B–5F). Also as expected, and as a control analysis, the decoding performance was at chance level when the classifier was trained in the fixation period and tested within the same and subsequent periods (Figure 5A).

When training the classifier on neuronal data during the cue period, decoding performance was close to chance level for subsequent time windows in the delay and response periods (Figure 5B). The exception was the first 500 ms of the delay period immediately after sample offset, which was probably due to a spill-over of neuronal activity at the transition between sample and delay periods. This suggests that the population codes between the sensory cueing period and the working memory period differ. Conversely, the cue period also could not be decoded above chance when the classifier was trained

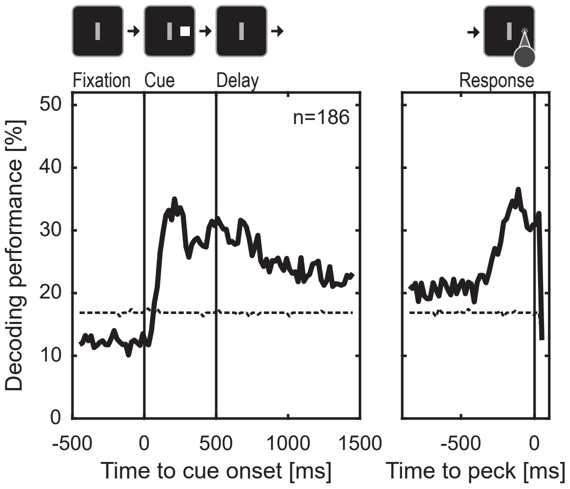

Figure 4. Neuronal Population Activity Encodes Cued Location throughout the Whole Task

Solid line represents cross-validation decoding performance of a k-nearest neighbor classifier. The dashed line represents the chance level (95th percentile of cross-validation with permuted labels). The left graph displays the decoding performance from fixation period until the first 1,000 ms of the delay. The right graph shows the decoding performance of data aligned to the peck on the screen (0 ms) during the response period.

in any of the delay and response windows (again, with the exception of the first 500 ms of the delay period).

Interestingly, however, the classifier trained in each of the three delay windows showed a significant decoding performance in all delay intervals (Figures 5C–5E). This indicates that the population code was stable across the delay period. Finally, a classifier trained on data of the time interval just before the peck also showed high decoding performance in the preceding delay periods (Figure 5F), arguably because of a spill-over of working memory information into the response period. In summary, this analysis suggests differing population codes between the sensory sample period and the subsequent working memory period. Within the delay period, however, the working memory code remained stable throughout the time-variable working memory period.

Activity Predicts Selections in Free-Choice Trials

In order to explore the significance of the NCL neuron firing rates for the crow's behavior, we introduced two-alternative free-choice trials. In such ambiguous trials, two opposite locations were cued simultaneously (either left and right, i.e., 180° and 0°, or top and bottom locations, i.e., 90° and 270°) (Figure 6A). The crows could pick either of these locations in the response period, and they were rewarded for either choice. We reasoned that if the activity of NCL neurons was behaviorally relevant, then the tuning of the neurons during the trial should allow a classifier to predict the crows' subsequent location choices. Both crows developed a response bias and tended to always chose the same of the two-alternative location. We trained a k-nearest neighbor classifier on the neuronal data of standard (i.e., non-ambiguous) trials in the cue, and the first, second, and last 500 ms of the delay period. After that, the classifier was used to predict the choice based on the neuronal data of ambiguous two-alternative choice trials. If the average performance based on the real data exceeded the 2.5 and 97.5 percentiles of randomly shuffled data, this performance was defined as significantly predicting the crow's choice. Figure 6B shows the data for ambiguous two-alternative choice trials in which the crows could choose between the top and bottom location (n = 25). The classifier was able to predict the later-chosen location significantly above chance in the cue period, the first, second, and last 500 ms of the delay period. Decoding performance was highest in the cue period and decreased across the delay but stayed above chance until the end of the delay period. Figure 6C displays the decoding performance in ambiguous two-alternative choice, in which the left and right locations were cued simultaneously (n = 31). The classifier was able to predict the crows' choice above chance level in the explored delay phases (first, second, and last 500 ms of the delay period) but failed to do so during the cue period. Decoding performance was highest in the first 500 ms of the delay period. Our data suggest different time courses for the two conditions. While the decision between top and bottom location was encoded both during the cue and delay periods, the decision between left and right was encoded only in the delay period. Overall, this analysis shows that the delay activity of NCL neurons reliably encoded the location the crow chose later in the trial across the whole delay period.

To further explore the functional role of NCL neurons in maintaining target location and predicting spatial choices, we compared population activity using a state space decoding analysis. In this analysis, the population activity over time is presented by a trajectory in three-dimensional space by the first three principal components. State space was calculated for the same populations used in the above analysis (n = 31 and n = 25). While the absolute positions of the trajectories in space are meaningless, spatial differences between the trajectories indicate coding differences. If the crow adopted the same behavioral strategy and the NCL population used the same code to represent the underlying memory content irrespective of whether regular or free-choice trials were presented, then the trajectories representing the same memory contents are expected to be very similar.

The trajectories resulting from this state space analysis are shown in Figure 7. Figure 7A shows the trajectories of population activity during regular trials with top (solid blue line) versus bottom (solid green orange) locations chosen, together with the trajectory during free-choice trials in which the crow chose the top target (dotted blue line). Figure 7B shows the trajectories of population activity during regular trials with left (solid magenta line) or right (solid green line) locations chosen, together with the trajectory during free-choice trials in which the crow memorized and later chose the right target (dotted green line). As expected for the crow's diametrically opposed memorized locations, population activity in regular trials diverged dramatically from cue period and throughout the entire delay period (solid blue versus orange in Figure 7A and magenta versus green trajectories in Figure 7B).

Interestingly, the trajectories derived for free-choice trials closely followed the trajectory of regular trials for identical memorized and later-chosen locations: when the crow

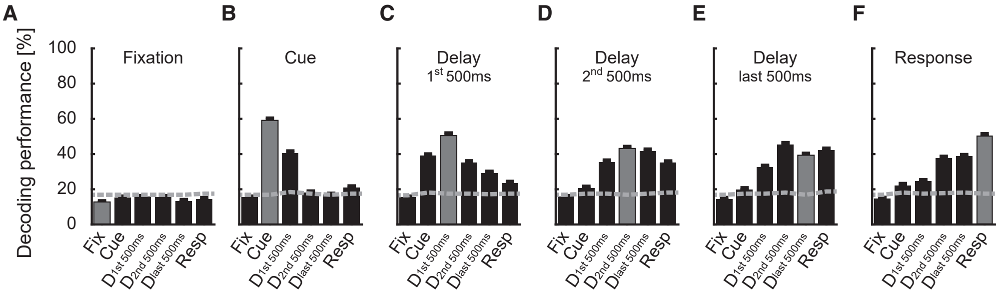

Figure 5. Exploration of Population Code across the Trial Periods

A k-nearest neighbor classifier was trained on data in a specific interval (gray bars in each diagram) and used to predict data in other epochs of the task. The classifier was trained on data during fixation (A), cue (B), first, second, and last 500 ms of the delay (C–E), and 500 ms before the peck (F). The gray bar indicates the cross-validation performance of a classifier tested within the trained interval (measure of cue information present in epoch). Black bars represent the prediction performance of the same classifier for other task epochs (measure of population code similarity between epochs). The dotted line represents the 95th percentile of classifications with permuted labels (chance level). Error bars represent the standard error of the mean (SEM).

memorized and finally pecked at the top location in free-choice trials (dotted blue line), the trajectory followed the one preceding top choices in regular trials (solid blue line) (Figure 7A). Similarly, when the crow memorized the right location in free-choice trials (dotted green line), the trajectory followed the one preceding right choices in regular trials (solid green line) (Figure 7B). This finding argues twofold: first, neuronal signals in free-choice trials and regular trials were virtually identical for the same behavioral representation by the crow, and second, the different memorized locations are reflected throughout the delay period to support the crow's future choice.

DISCUSSION

Over the past years, several functional similarities between the avian NCL and the primate PFC have been discovered [15, 19]. NCL neurons encode sensory [18, 35, 36] and cognitive [16, 17, 27, 37] variables and also participate in the execution of visually guided motor behavior [38]. While some studies have investigated neuronal correlates of working memory for objects in birds [26, 27, 39, 40], so far, none have addressed spatial working memory representations. We report that a large proportion of neurons signals and maintains visuo-spatial information, suggesting that the corvid NCL plays an important role in spatial working memory. Forty-one percent of all recorded neurons significantly encoded the memorized cue location. Almost 30% of all neurons were selective in both the cue and delay period, and their spatial tuning was significantly correlated. As evidenced by a classifier analysis, the population of all recorded NCL neurons maintained the spatial information of stimuli throughout the trial, thus bridging the temporal gap until a response was required.

NCL Activity Predicts Prospective Spatial Choice

The NCL neurons' activity is relevant for the crows' spatial behavior because in free-choice trials, the crows' prospectively chosen location could be predicted based on the tuned neurons' activity (Figure 6). This conclusion is also corroborated by the population state space decoding analysis. It showed that the population code throughout the delay period in free-choice trials and regular trials was virtually identical for the same choices made by the crow (Figure 7). This suggests that NCL neurons do not simply represent and store any visual information; rather, the NCL selectively represents information necessary to guide and control the crows' future behavior in space.

Such a selective storing of information that can also be witnessed in the primate prefrontal cortex (PFC) [41, 42] might be an important adaptation to the limited capacity of working memory [43]. Our results complement earlier findings in other cognitive domains that point to the corvid NCL as a pivotal brain center for cognitive control functions [16, 17, 19, 26, 27].

Balanced Representation of the Visuospatial Space in NCL versus PFC

In primates, spatial working memory has been studied extensively in the PFC with the oculomotor delayed-response (ODR) task, in which monkeys have to make a saccade to a briefly remembered spatial location [31, 44, 45]. Except for the responses required from the animals (eye movement in monkeys versus pecking in crows), the ODR is equivalent to the task we used in crows. Neurons in PFC readily encode spatial locations and maintain this information over delay periods [20, 31, 41, 44, 45]. In the PFC, more neurons are tuned to the visual hemifield contralateral to the recorded hemisphere [41, 44]. This contrasts our findings in the corvid NCL, in which an equal amount of neurons was tuned to both the left and right visual hemifields in both cue and delay periods (Figures 2E and 2F). This balanced representation of the visuo-spatial space is all the more surprising given that carrion crows have panoramic vision with only limited binocular overlap of maximally 37° of visual angle of the monocular visual fields [46]. In addition, and like all birds, they possess a complete decussation of the optic nerve, a purely contralateral projection of early visual information [47], and they lack a corpus callosum that connects the left and right endbrain hemispheres in mammals. This indicates major anatomical differences in the wiring of the main visual pathways between primates and birds [48], ultimately leading to a seemingly balanced representation of both visual fields in the NCL.

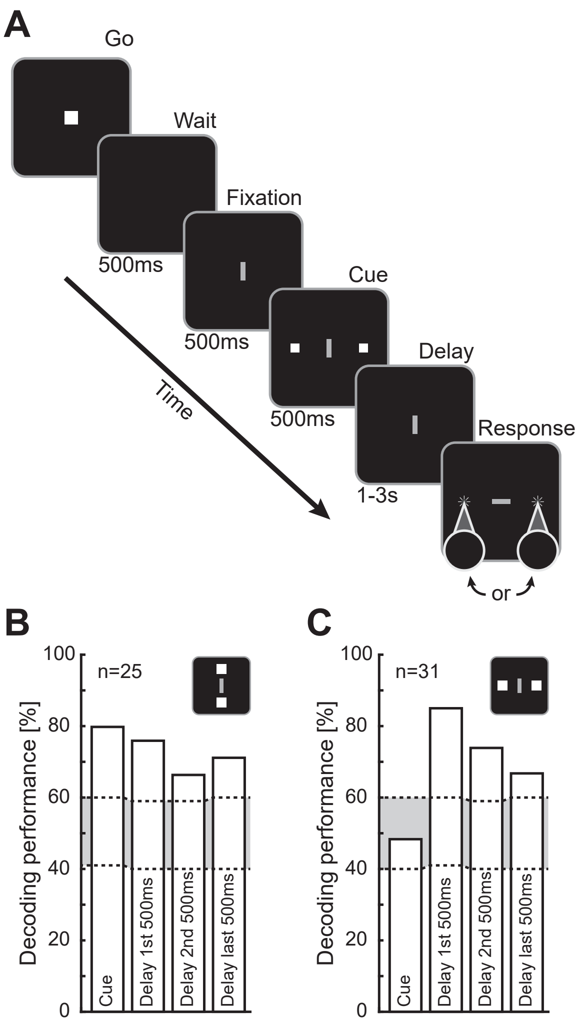

Figure 6. Task Layout and Classifier Decoding Performance for the Two-Alternative Free-Choice Trials

(A) Two-alternative free-choice protocol. The same protocol as main task (Figure 1A) but with two locations cued simultaneously during the cue period. The cued locations were either left and right (180° and 0°) or top and bottom (90° and 270°). During the response period, the bird was rewarded for choosing either of the cued locations.

(B and C) Choice prediction performance of a k-nearest neighbor classifier based on the responses of all neurons for the crow's top-bottom (B) and left-right (C) choices. The classifier was trained on neuronal data in the cue period and the first and second 500 ms of the delay period. The dashed line represents the chance level (2.5th and 97.5th percentile of prediction with randomly labeled trials).

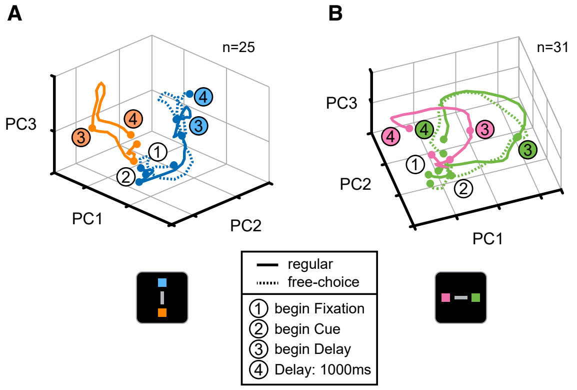

Figure 7. State Space Analysis Displays Similarity of Population Responses in Regular and Free-Choice Trials with the Same Goal

Development of population activity during free-choice and regular trials for cue locations top and bottom (A) and left and right (B). Solid lines represent the population activity trajectory during regular trials. The dotted line represents the activity during free-choice trials. The color of lines indicates the cued or chosen location (blue, orange, magenta, green for top, bottom, left, and right). For both the left-right and the top-bottom comparisons, the population activity of regular trials diverges after the beginning of the cue period and over the first second of the delay period. The population activity of free-choice trials resembles the activity of regular trials when chosen locations are identical.

The current study also highlights differences in working memory components found in the NCL and the primate PFC. Based on their firing rate increases with fast pecking reaction times, only a small proportion (8%) of the delay selective NCL cells were identified as motor preparation units; the vast majority of selective cells could be classified as storage units related to working memory. This is in stark contrast to findings in the monkey PFC, in which a majority of 55% of the cells could be linked to response preparation based on a very similar analysis [32]. It therefore seems likely that the avian NCL is less related to response selection processes than the primate PFC.

Sustained versus Dynamic Memory Code

NCL neurons showing sustained activity during memory delays are ideally suited to bridge the temporal gap between spatial cueing and response execution. Sustained activity has been reported as a neuronal correlate of WM since the early 70s [49, 50] and since then has been encountered in virtually all associative regions of the mammalian cerebral cortex, most notably, the prefrontal cortex (PFC) [31, 41, 51–53]. Sustained activity seems to be an evolutionarily conserved neuronal signature of working memory. It also exists in the endbrain of birds that do not have the sophisticated circuitry of a six-layered neocortex [16, 17, 25–27, 35, 39]. However, more recent studies in primates have emphasized that an additional code could be at work: many neurons show patterns of neural activity that are selective for only short periods of time during longer memory delay periods [54]. Analyses of neuronal population activity also revealed dynamic population coding phenomena associated with working memory tasks that were different from sustained activity [33, 34].

Our cross-temporal classifier analysis (Figure 3) suggests that the neuronal code for visuospatial working memory in the NCL is stable throughout the delay period because neuronal information extracted in one specific delay window enabled the classifier to predict performance in any other delay window. A likely explanation for this stability is the sustained firing of many memory-selective neurons such as the one displayed in Figure 2C. Of course, this does not preclude other codes from play a part; future research is needed to explore whether dynamic codes might complement the stable code to represent working memory contents in the avian endbrain.

In sum, the current findings together with previous insights [15, 19, 55] highlight the NCL as the corvid brain's central executive. Intelligence in birds is realized with an endbrain design that is radically different from the mammalian neocortex and developed independently via convergent evolution [56–59]. Comparative neurophysiological data in corvids and primates will help to decipher the general principles and evolutionary constraints for the design of clever vertebrate brains [60].

STAR★METHODS

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AUTHOR CONTRIBUTIONS

P.R. and A.N. designed the experiment. P.R. and M.E.K. conducted the experiments. P.R. analyzed the data. P.R. 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	bird V, bird W
Software and Algorithms		
NIMH Cortex	National Institute of Mental Health	c598; https://www.nimh.nih.gov/research/research-conducted-at-nimh/research-areas/clinics-and-labs/ln/shn/software-projects.shtml
MAP Data Acquisition System	Plexon	https://plexon.com/
MATLAB R2017a	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

LEAD CONTACT AND MATERIALS AVAILABILITY

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).

This study did not generate new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals

We used one 2- and one 4-year-old male carrion crows (*Corvus corone*) (bird V and bird W respectively) from the institute's breeding facility for the experiment. They were hand-raised and housed in social groups in indoor aviaries [28]. The crows 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

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 and to collect behavioral responses. Reward was delivered by an automated feeder below the touchscreen. The food reward consisted of food pellets (*Beo Special*, Vitakraft, Bremen or *NutriBird Beo komplett*, 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 birds were trained on a spatial delayed-response task including visual fixation (Figure 1A). The crow initiated a trial by positioning its head facing the monitor whenever a go-stimulus (white square, 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 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 500 ms (wait period). In the following fixation period (500 ms duration), a small vertical bar (0.33 × 0.65 deg visual angle) was presented as a fixation stimulus in the center of the screen. The size of the fixation target was close to the crows' visual acuity that is estimated to be 0.12 deg visual angle (or 8.4 cycles/degree) at a high luminance of 300 cd/m2 [61]. The crows were trained to observe this fixation stimulus throughout the following task periods and only respond when it changed orientation after an unpredictable time period. The fixation period was followed by the cue period (500 ms duration) in which one of eight different locations was cued by a white square (3.2x3.2° visual angle). These locations were arranged circular around the center of the screen, equidistant to the center (distance to center: 24° visual angle) and evenly distributed in angles of 45 degrees along the circular path (0°, 45°, 90°, 135°, 180°, 225°, 270°, 315°). The distance from one cue to the next was 18.4° visual angle. After the end of the cue period, the spatial cue disappeared and the delay period started with only the fixation target on an all-black background. The length of the delay period was varied pseudo-randomly between 1,000 ms and 3,000 ms (in steps of 500 ms). The end of the delay and beginning of the response period was indicated by a change in orientation of the central fixation bar from vertical to horizontal. After the fixation bar changed its orientation, the crow had to peck as fast as possible and no later than 900 ms after the change of the fixation bar at the previously cued location on the touchscreen. Trials with a reaction time longer than 900 ms were aborted without a reward and followed by a timeout of 1.5 s in which the beginning of a new trial was delayed as "punishment." Thus, the crows were discouraged from making head or eye movements and forced to closely pay attention to the fixation bar during the memory period by different factors: The small fixation bar close to the crows' perceptual threshold, the variable delay period, and response pecks under time pressure. The crows' overall correct performance of close to 100% (Figure 1B) further argue that they were fixating the fixation target throughout the delay period.

Pecks within ± 6° of visual angle (vertical and horizontal) around the center of the cued location were counted as correct responses and were rewarded by food. All pecks with a larger distance from the center of the cued location were counted as errors and were not rewarded. In addition, pecking errors were followed by timeouts. Pecking location errors between ± 6° and ± 8° of visual angle resulted in a shorter timeout of 1.5 s. Errors further away than ± 8°of visual angle from the target location resulted in 3 s timeouts. Both the cued location and the delay lengths were shuffled pseudo-randomly on a trial by trial basis by the computer running the task.

To explore the behavioral significance of spatial delay activity during this main task, we additionally presented crow W with two-alternative free-choice trials (Figure 4A). In these two-alternative choice trials, not one but two locations were cued simultaneously during the cue period. Either the locations left and right (180° and 0°) or the top and bottom locations (90° and 270°) were cued simultaneously. The crow could memorize and chose either of these locations to receive a reward in the response period. All other parameters were identical to the main task. Two-alternative free-choice trials were interleaved with the main task and occurred at a frequency of 10%.

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 in the medial part of NCL (*Nidopallium caudolaterale*) [29] by performing a craniotomy at 5mm anteriorposterior and 13mm mediolateral on the right hemisphere. This part of NCL, termed mNCL [29], is known to contain highly associative neurons [16–18, 26, 27]. 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). No obvious anatomical organization of location preferences was detected.

QUANTIFICATION AND STATISTICAL ANALYSIS

All analyses were performed in MATLAB. Values in main text and figures represent the mean ± SEM (standard error of the mean), if not stated otherwise. SEM was calculated as the standard deviation divided by the square root of number of samples.

Behavioral analysis

To measure the performance for normal trials we calculated the percentage of correct trials for each cued location on each recording day. For free-choice trials we calculated the percentage of choices to the top location (top-bottom-choice trials) or right location (left-right-choice trials). This measure allows to check for a bias toward always choosing the same location.

Neuronal location selectivity analysis

All cells that had at least 1Hz average firing rate and were recorded for at least 10 correct trials for each cued location were analyzed for this study. To analyze if a neuron selectively responded to different cued locations, we performed one-factorial analyses of variance (ANOVA; $p < 0.01$) with main factor "cued location" for the neuronal data on correct trials. For the cue period, selectivity was evaluated in a 500 ms window starting 100 ms after cue onset, to account for visual response latency of crow's NCL neurons [38]. For the delay period, selectivity was calculated over a 900 ms window starting 200 ms after the beginning of the delay period and reaching 100 ms into the choice period, again to account for the visual response latency of NCL neurons.

Neuronal preferred direction analysis

We calculated the preferred direction for each neuron in the cue and delay period. To that aim, we calculated the average firing rate across correct trials for each cued location. For each of the eight locations, an individual vector with a length corresponding to the average firing rate to this location was created. The preferred direction of a neuron was defined as the angle of the average vector resulting from vector addition of all eight vectors. To evaluate correlation between spatial preferences in the cue and delay periods, we calculated a circular-circular correlation between the preferred directions using the CircStat toolbox for MATLAB [30].

Differentiating storage and response neurons

We analyzed whether delay-selective neurons at the end of the delay showed neuronal activity related to the crows' reaction time [32]. For each recording session, we split all trials into fast reaction time and slow reaction time trials (50% each). Fast reaction times were defined as reaction times faster than the median of the session (median split). We then tested if the firing rate of a neuron was significantly increased during trials with fast reaction times compared to slow reaction times. Only trials for the preferred location and the neighboring locations of a neuron were analyzed. The preferred location was defined as the location closest to the calculated preferred direction of the neuron. As an example, if a neurons preferred direction was at 85°, the preferred location would be defined as 90° (top), and trials from locations 45°, 90°, and 135° would enter the analysis. All delay-selective neurons that were recorded for at least 20 fast and 20 slow reaction time trials were analyzed (112/120). We analyzed the last 500 ms of the delay period. A Mann-Whitney-U test ($\alpha = 0.05$) was calculated over the average firing rates of the analysis window to identify neurons with a significantly increased firing rate for trials with fast reaction times.

Population analysis for decoding cued location

To investigate if the location chosen by the crows could be predicted based on neuronal population activity, we used a k-nearest neighbor classifier and performed a 5-fold cross-validation. The k-nearest neighbor classifier creates a matrix of all neurons of a population and trials of different conditions. This matrix is used to create a n-dimensional space with t data points in it, with n being the number of neurons in the population and t being trials. Each of these t data points has a position in the n-dimensional space, according to the normalized firing rate of each neuron in the trial, and a label that is the cued location in the trial. The construction of the n-dimensional space is also referred to as training of the classifier. Using the n-dimensional space, the classifier can predict the label of a data point by assigning it the label of the k data points that have the shortest Euclidean distance. If these k data points have different labels, the most frequent label is used. In the 5-fold cross-validation, all data points are split up into five equal sized groups. Four of these groups are used to create the classifier matrix and predict the label of the remaining group. This process is repeated until all groups were predicted once. In doing so cross-validation prevents that a data point is used in the prediction of its own label and thereby the overestimation of classification power. The cross-validation performance is equal to the percentage of correctly predicted labels.

We used a sliding window approach (window of 100 ms and step size of 20 ms) to evaluate the cross-validation performance across the trial. Cells had to reach the general criterion described above and additionally had to be recorded for at least 20 correct trials in each location. To create the classifier matrix in an analysis window, we randomly chose 20 correct trials for each cued location of each neuron. These trials were combined to a $n \times 160$ matrix ($n = 186$), representing a population of pseudo-simultaneously recorded neurons. A 5-fold cross-validation was performed to calculate the decoding performance of the classifier ($k = 5$; 20 trials for each direction).

In order to be able to compare our results to chance level, we permuted the labels of the classifier 50 times and calculated the cross-validation performance for each permutation. This process was repeated 20 times, yielding 20 cross-validation performances for true labels and 1000 performances for the permuted labels. We averaged across the 20 true label performances to account for differences in trial selection. The average decoding performance was defined to be above chance level, if it was above the 95th percentile of the permuted data.

Stability of population code analysis

In order to analyze the consistency of selective encoding across time, we used a k-nearest neighbor classification while varying training and test time intervals. A classifier was trained over a 500 ms interval, and then used to calculate the inherent cue information in this interval using a 5-fold cross-validation. In addition, it was used to predict the cued location of data from other 500 ms intervals. The latter prediction was used to see if the code present in the training interval was similar to or different from other times during the protocol. The analysis of the data is similar to the k-nearest neighbor population analysis above ($n = 186$). Thus, only differences to the above analysis are described here. We compared neuronal data of fixation, cue, the first, second, and last 500 ms of the delay, and 500 ms before the peck in this analysis. The analysis windows of Cue period, the first, and second 500 ms of the delay were shifted by 100 ms to account for visual latency. For decoding performance inside the trained interval, we calculated a 5-fold cross-validation. As a chance level for this cross-validation we defined the 95th percentile of cross-validation performances with permuted labels. Qualitatively similar performance was obtained by leave-one-out classification. To calculate the prediction performance across intervals, we used the classifier to predict the labels of 20 randomly selected trials for each of the eight locations. As a chance level for this prediction analysis, we defined the 95th percentile of prediction performances using a classifier with permuted labels. To account for variability between trials, cross-validation and prediction was repeated 20 times, and permutation analysis 1000 times.

Prediction of choice in free-choice trials

We used a k-nearest neighbor classifier in two-alternative free-choice trials to determine whether neuronal activity encoded the spatial working memory of the crows' chosen location. Choice trials were only used and recorded in a subset of recording sessions with bird W. The animal developed a strong bias to always choose the top location (90°) in the top-bottom and the right location (0°) in left-right choice trials. We therefore only used choice trials with top and right choices for the following analysis in order to have a sufficient number of trials for this analysis. All cells in the analysis had to be recorded for at least 10 correct choice trials toward the respective location and 20 normal trials for the respective and opposite location each ($n = 25$ for top-bottom; $n = 31$ for left-right choice trials). We constructed a classifier based on the neuronal data of normal trials ($k = 5$; 20 normal trials for each direction). Then 100 choice trials were constructed by randomly drawing a choice trial from each cell. We used the classifier to predict the labels for all choice trials. Decoding performance was defined as the percentage of correct predicted choice labels.

In order to compare our results to chance level, we subsequently assigned random labels (choice or opposite location) to the choice trials and calculated the percentage of correct labeled trials. This process was repeated 1000 times, yielding 1000 decoding performances for correct labels and 1000 decoding performances for randomly assigned labels. We defined decoding performance to be above chance level, if the average decoding performance was outside the 2.5 and 97.5 percentile of the performances of randomly assigned labels. We analyzed the cue period in a 500 ms window aligned to cue onset as well as the first and second 500 ms of the delay period. All windows were shifted by 100 ms to account for visual latency.

State Space analysis

We performed a state space analysis on the neuronal population activity to see if free-choice trial activity was different from normal trial activity. All neurons that entered the analysis for prediction of choice in free-choice trials entered the principal component analysis (PCA). The PCA performs a realignment of the dimensional axes in the n-dimensional space that is created by the population activity of n neurons. The new dimensional axes are chosen to explain the maximum variance within the data. The variance explained by the first principal component is highest, followed by the second, and subsequent principal components (for a more detailed explanation see [62]). For left and right regular and free-choice trials the first three principal components captured 61.7% of the total variance, for top and bottom they explained 44.8% of the variance. Neuronal activity of all trials was smoothed with a 300 ms Gaussian kernel and activity was averaged over time bins of 50 ms.

DATA AND CODE AVAILABILITY

The datasets and code supporting the current study have not been deposited in a public repository because of further analyses, but are available by request to the Lead Contact, Andreas Nieder (andreas.nieder@uni-tuebingen.de).

Publication 2: Single neurons encoding spatial motor planning and execution in crow endbrain

Rinnert P, Nieder A (manuscript) Single neurons encoding spatial motor planning and execution in crow endbrain.

Single neurons encoding spatial motor planning and execution in crow endbrain

Abstract

Previous studies demonstrated an involvement of the avian endbrain area *Nidopallium caudolaterale* (NCL) in complex cognitive functions but also in the less cognitive execution of motor actions. However, the involvement of NCL in planning or preparation of such motor actions remained unknown. To address this question, we trained two carrion crows (*Corvus corone*) on a delayed response protocol, in which they had to plan movements to instructed spatial locations. We recorded the activity of single neurons in the NCL while the corvids solved that task. We found single neurons that prospectively encoded the side or specific location of the future target. The majority of these selective neurons preferentially encoded planned movements to the contralateral hemifield and did so also in absence of concrete instructions. In line with previous studies we also found neurons that encode the movement direction during movement execution. This study demonstrates the involvement of NCL in planning and execution of motor actions and suggests a pivotal role in motor control.

Introduction

In their natural habitat corvids as well as most animals are driven by goals that cannot be achieved in a single step. These problems are usually addressed with a sequence of planned actions. The goal of building a nest for example is a complex problem, that takes multiple single steps to complete: Lots of different materials have to be gathered one by one in the correct order and combined precisely to end up with a robust nest for the offspring. Often the planned sequence of steps to achieve a goal has to be reevaluated and adapted flexibly. When preying on insects, for example, a single planning of movements to catch the prey might not suffice: Once the insect moves, all motor plans have to be reevaluated and adjusted. For this readjustment of motor plans the animal has to integrate new sensory information about the target location with the overall goal and current state of the animal. Both examples highlight the necessity that for achieving a complex goal, it has to be split up in single goal directed behaviors i.e. motor plans. If successful, each goal directed behavior brings the animal one step closer to achieve the overall goal. In order to be able to overview and to adapt, the overall goal, planned goal directed movements and the current state of the animal need to be monitored closely by a central executive instance.

On neuronal level, previous research highlighted the involvement of one particular brain area, the *Nidopallium caudolaterale* (NCL), in goal directed behavior and integration of multimodal information (Veit and Nieder, 2013; Moll and Nieder, 2017). The anatomy of the avian brain puts the NCL atop of the cortical hierarchy, with reciprocal connection to all secondary sensory areas, as well as motor output structures (Divac et al., 1985; Güntürkün, 2005; Nieder, 2017). Studies found that NCL neurons encode and maintain sensory information that is relevant for completion of a learned task (Veit et al., 2014; Johnston et al., 2017; Wagener and Nieder, 2017). In a previous study we found that single neurons maintained the spatial location of objects in working memory during a memory delay (Rinnert et al., 2019). The spatial location of a target is one key feature necessary for the planning of movements towards a target. In addition, two studies found that NCL neurons also encodes the actual direction of movement of the animal during execution (Lengersdorf et al., 2014a; Veit et al., 2015a). Thus, we know that NCL represents behavioral relevant sensory information and at least monitors the execution of movements. So far, the involvement of NCL in planning of movements was unclear.

In the present study we wanted to investigate, if NCL is also encoding planned motor responses. We trained two carrion crows on a delayed response protocol, that did not require the maintenance of information in working memory. The animal was trained to prepare a response to one of eight different locations on a screen. Importantly, the information about the later target was present throughout the whole planning period until the response had to be made. Therefore, a maintenance of spatial target information in working memory was not necessary in our protocol. Single neurons in NCL encoded the planned movement direction and the direction of movement during movement execution. While neurons encoding planned movements had a bias for contralateral targets, this was not found during movement execution.

Results

Two male carrion crows were trained on a spatial delayed response protocol, in which they had to prepare movements to one of eight circularly arranged target locations (Figure 1A, target locations see 1B). In each trial during the cue period, two grey frames introduced two possible target locations on a touchscreen. The possible target locations were horizontally aligned and there was always one location on the left and one on the right side of the screen. In the planning period, a rule cue in the center of the screen instructed the animal to plan and later execute a movement response to either the left or right target location. Movement execution was instructed after a variable planning time between 1s and 3s, by vanishing of the rule cue. A rapid response to the instructed target within 800ms was rewarded. The variable time and short response window forced the animal to prepare a movement in advance. Importantly, a memorization of the instructed location was not necessary, because rule cue and target location were presented continuously throughout the planning period. The rule cues instructing left or right, differed either in color or shape (color or shape cues, Figure 1B). In 20% of the trials we presented an ambiguous rule cue, that instructed neither of the target locations. In these trials’ responses to either of the targets were rewarded randomly half of the time.

Behavior

Both birds performed well above chance level for all recording days. The average performance across all eight target locations was above 80% for all recording sessions (Bird W: 88.7%±4.6; Bird V: 98.6%±2.3). Performance split up for each side was significantly above the chance level of 50% (Left side: 86.8%±5.9 and 97.8%±4.0; Right side: 90.8%±3.9 and 99.6%±0.8 for bird W and bird V respectively; binomial test $p<0.05$; Figure 1C). Also the performance for each type of rule cue was significantly above chance level (Color cues: 89.9%±5.5 and 98.9%±1.8; Shape cues: 87.7%±4.1 and 98.4%±3.8 for bird W and bird V respectively; binomial tests $p<0.05$; Figure 1C). It took bird W on average 516ms and bird V 507ms to respond to the instructed location.

In ambiguous trials both birds developed a bias to always choose the same side. The idea behind this condition was to introduce ambiguity about the instructed side that should result in a random and even distribution of choices to both sides. To accomplish a uniform distribution of choices, the ambiguous cue was adapted according to the previous day bias in ambiguous conditions. Both birds tended to choose always the same side in ambiguous conditions within a session. The bias for a side was changing between sessions, as the ambiguous cue was altered in color or shape. Bird W had a strong bias towards choosing the right side in ambiguous conditions, while the bias in bird V changed more flexibly between days (average percent left side chosen over all recording sessions 23.6%±19.6 for bird W, and 42.9%±37.7 for bird V).

Neuronal data

Single neurons in NCL encode side and/or location of the later target during planning

We recorded the activity of 408 single neurons in the left NCL. Each of these neurons was recorded for at least 20 trials per rule cue and had at least 1Hz average firing rate. The activity of neurons was modulated by individual target locations or all target locations on one side. The example neuron in Figure 2A increases its activity in trials in which a target location was on the left side (left panel). The increase of activity is present irrespective of the rule cue type that instructs the target side (center panel). Hence, this selective activity cannot be explained by visual responses to a specific rule cue. When neuronal activity is averaged over the begin of the planning period (shaded area), the firing rates for all positions on the left side is virtually identical (right panel and polar plot). The neuron is therefore not differentiating between different target locations within the left side but is encoding trials with target on the left side versus target on the right side. Figure 2B shows a different example neuron. The activity of this neuron is higher during the planning period for trials in which a target on the right side is instructed (left panel). Also, this neuron is not differentiating between the type of rule cue that instruct a side (center panel) and is therefore not encoding the visual features of the rule cue. When averaging activity for the eight different target locations, activity for all target locations on the right is higher compared to target locations on the left side (right panel and polar plot). In contrast to the first example, however, this neuron shows differential activity for the target locations within the right side: The activity for target locations 2 and 3 is higher than for locations 1 and 4. This neuron therefore seems to differentiate between a target on the left and a target on the right and additionally has a preference for specific target locations.

Many NCL neurons encode the target side or location during planning

To quantify the involvement of NCL neurons in encoding of target side or location on a population level, we used analyses of variance (three-factorial nested ANOVA with factors target side, target location, and rule cue type). Data was analyzed in a 900ms window starting 100ms after beginning of the planning period (see shaded area in Figure 2A and 2B). We excluded all neurons with a selective factor rule cue type, because these neurons were affected by the visual features of the rule cues. The activity of 28.7% (117/408) of all recorded neurons was modulated either by the target location, the target side, or both ($p<0.05$ for factors side and/or position, $p>0.05$ for factor rule cue type). More than half (61/117) of this selective subpopulation was only modulated by the target side and not individual target locations, similar to the neuron in Figure 2A. These neurons encoding the target side, will be called side selective in the following. The rest of the neurons were either modulated only by individual target locations (33/117) or a combination of target side and location (23/117), like the neuron in Figure 2B. Neurons with only target location selectivity will be called location selective, while neurons with selectivity for both target location and side will be called location and side selective.

Neurons selective during planning period prefer targets on the contralateral side

We calculated the preferred direction for each neuron selective in the planning period (n=117). For each target location a vector with the length of the average firing rate for all correct trials to that location and the direction of the target location was calculated. The preferred direction of a neuron was defined as the direction of the vector resulting from vector addition of all target location vectors (see arrow in polar plots in Figure 2A and 2B). The preferred direction of all planning selective neurons is shown in Figure 2C. Significantly more neurons preferred target locations on the right side (88/117 or 75% preferring target locations on the right side; binomial test $p<0.05$). Because we recorded neuronal activity in the left NCL in both birds, these neurons preferred locations in the contralateral hemifield.

Neuronal planning activity predicts later chosen target in ambiguous trials

We observed neurons that displayed activity during the planning period in ambiguous trials, that resembled the activity during normal trials with the same target. The example neuron in Figure 3A has an increased firing rate for normal trials in which the target is on the right side. In ambiguous trials in which the bird chose the target location on the right side, the neuronal activity of this neuron resembles the activity during normal trials with the target on the right. Moreover, the activity during ambiguous trials with later response to the target on the left side resembles that of normal trials with target on the left side. In addition, when neuronal activity for this neuron is split up for individual target locations, the neuronal activity during ambiguous trials resemble that of normal trials with the same target location. This indicates that neuronal activity could be predictive of the later target location in trials with ambiguous rule cue information.

To evaluate if the activity during ambiguous trials is predictive of the later chosen side or location, we analyzed population activity using a support vector machine (SVM) classifier. If the selective activity in the planning period was related to the motor target side or location, the activity should not differ substantially between normal and ambiguous trials with the same target location. Hence, an ideal observer should be able to predict the choice during ambiguous trials on basis of the neuronal activity. We trained an SVM classifier on the neuronal population activity of normal trials and used the classifier to predict the chosen target side or location during ambiguous trials. We defined prediction performance to be significantly above chance level, if the average prediction performance was above the 95th percentile of predictions using a classifier trained on permuted data. Figure 3B shows the prediction performance for target side in ambiguous trials. The classifier was able to predict the later choice of target side significantly above chance level. Moreover, also the prediction of individual target locations in ambiguous trials was significantly higher than chance (Figure 3C). This analysis shows that neuronal activity during ambiguous trials did not differ from normal trials with the same target side or location.

As an additional line of argument, we performed an AUROC analysis, to test whether side-selective neurons showed the same selectivity also during ambiguous trials. AUROC is a measure derived from signal detection theory, that describes the discriminability of two distributions (Green et al., 1966). We calculated an AUROC value in normal trials for each side selective neuron (84/408) and an AUROC value in ambiguous trials for all side selective neurons that were recorded for at least 5 choices for each side in the ambiguous condition (35/84). Values higher 0.5 represent a preference for the same side as in normal trials, while values lower 0.5 indicate a change in preference. The preferred side was defined by the preferred direction in normal trials categorized into left or right. AUROC values close to 1 or 0 indicate a strong discrimination between the sides, while a value of 0.5 indicates no difference between the firing rates for left and right. For normal trials all AUROC values were above 0.5, due to side selectivity and the definition of the preferred side (Figure 3D). The mean AUROC was lower in ambiguous trials compared to normal trials (0.55 for ambiguous and 0.62 for normal trials). Nevertheless, the mean AUROC in ambiguous trials was significantly higher than 0.5 (Wilcoxon signed rank test, $p<0.05$). Also this analysis indicates that neurons show the same kind of target side selectivity in both normal and ambiguous trials.

Single neurons encode target location during movement execution

The activity of single neurons was modulated by target location around the time of movement execution in the response period. Figure 4A shows the activity of an example neuron before and after a peck on the screen was registered. Shortly before the birds' peck on target location 7 and 8, this neuron increases its firing rate abruptly. While there was also a slight increase for pecks on target location 6, there was virtually no increase in firing rate for all other target locations. Hence, this neuron seems to be active during movements towards specific target locations in the response period.

A large proportion of neurons encode target location during movement execution

To investigate selective activity during movement execution in the response period on population level, we performed analyses of variance (4-factorial nested ANOVA with factors target side, target location, rule cue type, and planning period length). To capture the time of movement towards the target location, we analyzed data in a 350ms window ending 50ms before a peck on the screen. Neurons with a significant factor for rule cue type or planning period length were excluded because these neurons were encoding visual rule cue features or exhibited timing related activity. Nearly forty percent of all recorded neurons (38.7% or 158/408) were modulated by the target location or the target side during movement execution in the response period ($p<0.05$ for factors side and/or position, $p>0.05$ for factor rule cue type and planning period length). The number of selective neurons was about evenly distributed between neurons selective for only the factor target side (49/158 side selective neurons), only the factor target location (53/158 location selective neurons), or both target side and location (56 location-and-side selective neurons).

No bias in preferred direction during movement execution

We calculated the preferred direction for all neurons selective for factors target location or side during movement execution (158/408) in the same way as for the planning period. The preferred directions for all neurons can be seen in Figure 4B. Neurons were tuned to virtually all directions. In contrast to the neuronal population selective during the planning period, neurons during movement execution were about evenly distributed to both locations on the left and right, respectively the ipsi- and contralateral hemifield (73/158 or 46% preferring target locations on the right side; binomial test $p>0.05$).

Preferred directions in planning and response are correlated

A subpopulation of neurons was selective in both the planning and response period ($n=50$). To evaluate if the encoded locations were correlated, we calculated a circular-circular correlation between the preferred directions of these neurons (Berens, 2009). Neurons with selective activity in planning and response period, showed a significant correlation of their preferred directions ($p<0.05$, $\rho_{cc}=0.31$).

Sharper tuning of neurons during movement execution compared to planning

We noticed that neurons selective in both planning and response period tended to be tuned more precisely to single target locations during movement execution in the response period compared to their tuning in the planning period. Figure 5A shows one example neuron with its response profile during the planning period (left polar plot) and during movement execution in the response period (right polar plot). While this example neuron shows selective activity for target locations around target location 1 during both periods, the tuning is distinctly more sharp during movement execution. We wanted to analyze if this effect of sharper tuning during movement execution was a general effect in our population of selective neurons. As a measure for the tuning sharpness of neurons we calculated the Kappa κ of a von Mises distribution for all selective neurons in the planning and response period (Berens, 2009). A von Mises distribution is the circular equivalent of a gaussian distribution, with Kappa κ as an equivalent for the standard deviation σ. Large values of κ indicate sharp tuning to distinct locations, while a low κ indicates broad tuning. First, we compared the neurons selective in both periods of the protocol (Figure 5B) and found that κ was significantly larger during movement executions ($n=50$; Mann-Whitney-U-test $p<0.05$). Also in the remaining population of neurons selective during either planning or response period, κ was significantly larger in the population of neurons selective in the response period (Figure 5C; $n=67$ for planning and $n=108$ for response period; Wilcoxon-signed-rank-test $p<0.05$). These analyses show that neurons that are selective during planning and response and selective neurons in general are tuned more narrow during the response period.

Shift of target side and location encoding during planning to only target location during response

To further compare the encoding of target location and side during planning and response, we calculated a confusion matrix for SVM classifier trained to predict the target location during planning and response period. Both the classifier trained on neuronal data of the planning and response period, were able to predict the instructed target location significantly above chance level (Figure 5D and 5E right panel). The average decoding performance for data of the planning period was around 40%, while the decoding performance for the response period was above 70%. This indicates a more precise encoding of target locations during the response period and is in coherence to the significantly sharper tuning of neurons during that period. In a next step we evaluated the misclassifications of the classifier using a confusion matrix (Figure 5D and 5E left panel). In a confusion matrix the predictions of the classifier are averaged depending on the true labels, resulting in an 8x8 matrix for the eight possible target locations. A large proportion of correct predictions would result in a confusion matrix with a diagonal of high predictions, where the predicted labels match the true labels. When analyzing the confusion matrix for the planning period, we see a stronger diagonal for the first four target locations compared to the last four (Figure 5D, left panel). This is equivalent to a lower decoding performance for target locations on the left side (target locations 5 to 8) compared to target locations on the right side (target locations 1 to 4). The higher decoding performance for targets on the right side is in coherence with the larger number of selective neurons for that side or locations on that side during the planning period. Focusing on the misclassifications of the classifier, it is salient that errors in prediction of target location occur almost exclusively within a side. Trials with target location 1 on the right side are for example confused with target locations 2 to 4 more often than with target locations 5 to 8 on the opposite side. This indicates neuronal activity representing the target side during the planning period. In comparison, the confusion matrix of the classifier trained on data from the response period, displays a strong diagonal in coherence with the high overall decoding performance (Figure 5E, left panel). In contrast to the planning period, we do not see higher misclassification rates within a side compared to across sides. If misclassifications occur, they are mostly to neighboring locations, indicating a true spatial encoding of target location. In summary, this analysis shows encoding of both target side and location information during planning and encoding of only target location encoding during the response period.

Discussion

In recent years, the high-level association area NCL gained importance in research about cognitive functions in birds. Previous studies showed an encoding of sensory visual information (Veit et al., 2014; Johnston et al., 2017; Wagener and Nieder, 2017; Rinnert et al., 2019) and maintaining of spatial and object information in working memory (Veit et al., 2014; Rinnert et al., 2019). Other studies showed the involvement in learning and maintaining of cross-modal associations and complex rules (Veit and Nieder, 2013; Moll and Nieder, 2015; Veit et al., 2015b). While two studies also showed encoding of movement directions during movement execution, the involvement of NCL in the planning of such responses is unclear. We report neurons in NCL that encode the target of a motor response to the contralateral hemifield while the animal has to withhold execution of the movement. These results demonstrate an involvement of NCL in the planning of movements. More than a quarter of all recorded neurons encoded either precise target locations or more broadly the side of the later target. Classifier analysis showed that the selective activity was not depending on the rule cue instructing the target location but on the location of the target. In addition, this study also shows the large involvement of NCL in encoding of movement direction during execution. While the population tuning during planning of a movement is broader, encompassing multiple target locations, the tuning during movement execution is more precisely encoding the exact target location.

NCL activity during planning encodes the motor plan

More than every fourth NCL neuron exhibited selective activity for target locations during the planning period. Because we excluded all neurons with selectivity for a specific rule cue type, these neurons were not exhibiting visual sensory encoding of the rule cue. In contrast to previous studies, the protocol in this study did not include a memory delay and all information was always present on the screen. Hence, information did not need to be maintained in capacity limited working memory. This rationale was also previously used to exclude maintenance in working memory (Funahashi et al., 1991, 1993; Markowitz et al., 2015). On the other hand, a variable planning period length and short response time window ensured that the bird had to prepare a movement before the begin of the delay period. A classifier was able to predict, the chosen target side and location also during ambiguous trials (Figure 3B and 3C). This corroborates that the selective activity during planning was related to the later target location and not visual properties or rule information. Veit and Nieder (Veit and Nieder, 2013) found that NCL neurons encode rule information, which could also explain our selective activity. Although the rule cue in our study instructed left side or right side, this cannot explain neurons selective for individual target locations within a side. In addition, we found the majority of neurons to prefer target locations on the contralateral side, and there is no reasonable explanation for a population of neurons encoding only one of two possible rules. Therefore, the most parsimonious explanation for our data is an encoding of the upcoming motor goal.

In a previous study Lengersdorf et al. (2014a) trained pigeons to prepare movements to a left or a right key, depending on the grayscale value of a sample stimulus. Brighter grayscale values instructed the left, darker values the right target location. They found NCL activity that was related to the later response direction or side. The authors interpreted this as neurons conveying a motor plan to the left or right. However, the activity could also be explained by encoding of sensory visual information, because stimuli instructing left and right varied only in their luminance. Nevertheless, this study is to our knowledge the first study to suggest a representation of motor plans in NCL, based on single neuron activity. Our study circumvented this problem, by introducing rule cues that differed in either color or shape. All selective neurons in the present study could therefore be tested not to encode visual rule cue information, ruling out this possible confound. Interestingly, Lengersdorf et al. (2014a) did not find a preference of NCL neurons for encoding the movements to the contralateral side, which is in contrast to the present study. The involvement of NCL in the motor domain was also suggested by lesion studies in pigeons, that found an impairment in the execution of learned behavior after inactivation of NCL (Lissek and Güntürkün, 2004, 2005; Helduser and Güntürkün, 2012; Helduser et al., 2013; Lengersdorf et al., 2014b). The involvement of NCL in motor planning shown in the present study also fits NCLs connection with motor related areas (Kröner and Güntürkün, 1999).

A representation of motor plans in high associative brain areas is also known from the well-studied mammalian PFC, that is thought to be the functional analog to the avian NCL (Divac et al., 1985; Güntürkün, 2005; Nieder, 2017). As an example the study of Markowitz et al. (2015) found neurons in monkey PFC that maintained spatial information in working memory, while other neurons encoded the response direction of an upcoming saccade. Also several other studies found PFC neurons encoding the plan of an upcoming response for both eye and limb movements (Niki and Watanabe, 1976; Funahashi et al., 1993; Asaad et al., 1998; Hasegawa et al., 1998; Takeda and Funahashi, 2002).

NCL neurons encode movement direction during execution

While the bird was executing the planned movement towards the target location, nearly forty percent of all recorded neurons encoded the movement direction (Figure 4). In contrast to the planning period, the population of neurons was evenly tuned to virtually all directions, without a bias for a side. The large proportion of selective neurons allowed a classifier to predict the target of the movement with a performance of more than 70% (Figure 5E). The high resolution encoding of movement directions in single cells and the large proportion of selective neurons suggest an important role of NCL in encoding of movement directions.

The encoding of movement direction during execution in NCL single neurons was also shown by Veit et al. (2015a), in a study with crows trained on a delayed match to sample protocol. Similar to our study, a large fraction of single neurons encoded the movement direction while the bird executed a movement to one of four possible target locations. In coherence to our study also Veit et al. (2015a) found no population preference for any direction. Also in the aforementioned study by Lengersdorf et al. (2014a), a subset of NCL neurons encoded the movement to either the left or right target during the movement. While both studies showed the involvement of NCL in encoding of motor actions, our study extends this knowledge by showing neurons that differentiate between movements to eight different target locations.

Also neurons in the mammalian PFC encode movement direction around the time of execution. Takeda and Funahashi (2002) trained monkeys on a rotatory oculomotor-delayed response task. A quarter of the recorded PFC neurons encoded the saccade direction either immediately before or after the saccade. Similar results of PFC neurons encoding the saccade direction around time of execution were also reported in other studies (Funahashi et al., 1991; Asaad et al., 1998; Hasegawa et al., 1998). Takeda and Funahashi (2002) proposed the encoding of movement direction during execution in PFC as a potential termination signal for working memory activity. This explanation could also explain the selective activity found in the avian NCL, that is – like PFC – involved in working memory (Veit et al., 2014; Rinnert et al., 2019).

Transition from categorical side encoding during planning to precise location during execution of movements

Comparing the tuning broadness of single neuron during planning and execution of movement, we found an increase in tuning sharpness during movement execution (Figure 5A to C). This is also shown in the population classifier analysis in Figures 5D and 5E, where we see an encoding of both target side and location during planning and a strong encoding of target location without side during execution. Both analyses point to a change in spatial tuning precision from a planning stage without the need for precision to the actual execution, where high precision is essential. The significant correlation in preferred directions of neurons selective during both planning and execution indicates that the tuning can be compared directly. This subset of neurons might be the link between the planning and execution of movement.

Conclusion

While previous studies found an involvement of NCL neurons in encoding sensory visual and high-level cognitive correlates (Veit and Nieder, 2013; Veit et al., 2014; Ditz and Nieder, 2015, 2016; Wagener and Nieder, 2017; Rinnert et al., 2019), the present study highlights that NCL is also engaged in planning and execution of responses. We therefore close the loop and find NCL involved in all steps of a perception action cycle, with encoding of sensory information (Veit et al., 2014, 2015b; Ditz and Nieder, 2016; Johnston et al., 2017; Wagener and Nieder, 2017; Rinnert et al., 2019) that is maintained and integrated (Veit et al., 2014; Moll and Nieder, 2015, 2017; Rinnert et al., 2019) resulting in the planning of an appropriate behavioral response and the execution of this response (Lengersdorf et al., 2014a; Veit et al., 2015a). In conclusion, the NCL seems to represent all information important for the animals' behavior and would be perfectly suited as a central executive instance monitoring and supervising the system.

Methods

Animals

We used a two- and a one-year old carrion crow (*Corvus corone*) from the institutes breeding facility. Both animals were hand-raised and housed in social groups in indoor aviaries (Hoffmann et al., 2011). The animals were on a controlled feeding protocol during training and recording days. Body weight was measured daily. Food was given as reward during the experiment, or, if necessary, after. 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.

Surgery and Recordings

Surgery was performed under general anesthesia with a mixture of ketamine (50mg/kg) and xylazine (5mg/kg). The animals were placed in a stereotaxic holder and craniotomy was performed to target the medial part of NCL (Sen et al., 2019) with coordinates 5mm anteriorposterior and 13mm mediolateral in the left hemisphere. Two custom-build micro drives with four electrodes each (2MΩ, Alpha Omega Co.) were implanted at the craniotomy. In addition, a miniature connector for the headstage and small holder for the reflector were implanted. In each recording session electrode position was adapted until good neuronal signal was detected on at least one channel. Neurons were not pre-selected for involvement in the task. Single neuron separation was done offline (Plexon Offline Sorter, version 2.6.2).

Experimental setup

Experiments were conducted in a darkened operant conditioning chamber. The bird was perched in front of a touch screen (ART development MT1599-BS), that was used for stimulus presentation and to record behavioral responses. Food reward was delivered by a custom-built automated feeder below the touch screen. Food reward consisted of food pellets (*Beo special*, Vitakraft, Bremen) and mealworms (*Tenebrio molitor* larvae). Additional feedback was delivered by a lamp on the feeder and speakers (Lasmex S-03) located behind the touch screen. An infrared light barrier in front of the screen detected, when the bird was positioning its head in front of the screen. A CORTEX system (National Institute of Mental Health) was used to carry out the experiments and collect behavioral data. Neuronal data was recorded using a PLEXON system (Plexon Inc., Dallas, Texas).

Behavioral protocol

The birds were trained on a spatial delayed response protocol (Figure 1A). A go-stimulus (white square, 3.3x3.3 deg visual angle) instructed the bird to move its head into the light barrier to start a trial. If the head was moved out of the light barrier before beginning of the response period, the trial was aborted. Each trial started with a 300ms cue period, in which two grey frames (4.8x4.8 deg visual angle) introduced two possible target locations on the touchscreen. There were eight possible target locations, that were circularly arranged around the center of the screen (Figure 1B). In each trial the two possible target locations had the same vertical position (i.e. horizontally aligned), so that there was always one possible target location on the left and one on the right side of the screen. In the planning period a rule cue appeared in the center of the screen, in addition to the possible target locations. The rule cue instructed the bird to later perform a movement to either the left or right target location. The rule cues for left and right differed in either their color (color cues, 0.2x0.2 deg visual angle), with a red square instructing the left side and blue square instructing the right side, or their shape (shape cues, 0.2x0.6 and 0.6x0.2 deg visual angle), with a horizontal bar instructing the left side and a vertical bar instructing the right side. The length of the planning period was pseudo-randomly varied between 1 and 3 seconds (500ms steps). In the response period, a rapid peck to the instructed target location had to be performed within 800ms, to obtain a reward. Responses after 800ms or to the wrong target location resulted in abort of the trial without reward. The begin of the response period was indicated by a vanishing of the central rule cue. Important in this protocol was, that during the planning period both rule cue and target information were presented continuously, so that there was no need to memorize the target location. In addition, the variable length of the planning period and the short response window, made it impossible to predict the timepoint of the instructed response and made preparation beforehand necessary.

In twenty percent of the trials we introduced so called ambiguous rule cues, to compare neuronal activity during uncertain target location information. These ambiguous rule cues differed from the normal rule cues in color and shape and were therefore ambiguous in their instructive meaning. In the response period, choices to either target location were rewarded randomly in 50% of the trials. Ideally this should have resulted in random choosing of left and right target location from trial to trial, with equal distribution of choices to both sides. Due to individual biases in both birds, choices within a session were to a large fraction to the same side. To achieve a cue with an ambiguous meaning to the bird, we varied the color or shape of the ambiguous cues based on the previous day bias. An example for an ambiguous cue was a grey square, that could be interpreted as a color rule cue without color information or a shape rule cue without shape information.

Calculating the preferred direction for single neurons

To calculate the preferred direction for a neuron, we calculated the average firing rate during correct trials for each target location individually. For each target location a vector with the length of the average firing rate and the direction of the target location was created. The preferred direction of a neuron was defined as the direction of the vector resulting from vector addition of all target location vectors.

Support vector machine (SVM) prediction of target in ambiguous trials

We trained support vector machine (SVM) classifier on neuronal population activity of normal trials, to learn if also the activity during ambiguous trials was predictive of the later chosen target side or location. During training of an SVM classifier, the classifier creates a population of pseudo-simultaneous recorded neurons and tries to create boundary vectors separating the to be classified groups with the largest margin. For prediction of target side, all neurons with at least 5 ambiguous choices to each side and 40 normal trials for each side entered the analysis (n=248). For prediction of target location, all neurons with at least 2 ambiguous choices to each target location and 10 normal trials for each location entered the analysis (n=104). We analyzed average neuronal activity during the first 1000ms of the planning period, offset by 100ms to account for visual latency. Each classifier was trained on m randomly chosen normal trials of each neuron for each group (m=40 for target side and m=10 for target position). We used the LIBSVM library (version 3.23) (Chang and Lin, 2011) with all parameters set to default for training of the classifier. The default kernel for creation of boundaries is a radial basis function, but we also obtained qualitatively similar results using a linear kernel. For prediction of target side or location in ambiguous trials we randomly drew a randomly chosen ambiguous trial of each neuron for each group to create pseudo-population activity during ambiguous trials. This process was repeated 100 times resulting in 100 ambiguous test trials for each group. The prediction performance was defined as average of correctly predicted ambiguous trials. To compare the results to chance level, we trained another classifier with permuted trial labels. This classifier was used to predict the 100 test trials, resulting in a prediction performance under conditions without neuronal selectivity. The process of training of classifiers and predictions of test trials was repeated 100 times to account in differences in trial selection. Prediction performance was defined to be above chance level, if the average was above the 95th percentile of performances derived with the permuted classifier.

AUROC comparison of side-selective neurons in normal and ambiguous trials

The AUROC (area under the receiver operator characteristic) is a measure for the overlap of two distributions that is derived from signal detection theory (Green et al., 1966). The AUROC can take on values from 0 to 1. A value of 0.5 indicates a complete overlap of the distributions, while values of 0 or 1 indicate a complete separation of the distributions. In other words, a value of 0 or 1 means that all values of one distribution are higher than the values of the other distribution. We calculated an AUROC value of preferred versus non-preferred side during normal trials for all side-selective neurons (n=84). The preferred side was defined by the preferred direction categorized into left and right. Average firing rates of a 1000ms window starting 100ms after the begin of the planning period were analyzed. Next, we calculated the AUROC value during ambiguous trials for all side-selective neurons that were recorded for at least 5 ambiguous trials for each side (35/84). For this analysis we defined the preferred direction based on the preference in normal trials, so that values above 0.5 represent preference for the same side during normal and ambiguous trials.

Support vector machine (SVM) confusion matrix analysis

In this analysis we trained a support vector machine (SVM) classifier on the population activity of a subset of normal trials, to predict the labels of another test set of normal trials. Planning and response period were analyzed separately. We used the LIBSVM library (version 3.23) (Chang and Lin, 2011) with all parameters set to default for training of the classifier. For the planning period, we analyzed a 1000ms window starting 100ms after begin of the planning period, to account for visual latency. For the response period, we analyzed neuronal activity in a 350ms window ending 50ms before the peck, accounting for the time of movement execution. All neurons, that were recorded for at least 30 trials for each target location entered this analysis (n=175). We performed a five-fold cross-validation analysis on a classifier fed with 30 randomly chosen normal trials of each target location for each neuron, resulting in 240 or 30x8 data points. In a five-fold cross-validation, the dataset is split up into 5 equal sized parts of data points. In the next step the classifier is trained on four parts and tested on the remaining part. This process is repeated until all parts were predicted once. In this way cross-validation prevents an overestimation of classification performance, by ensuring that the data used for testing of the classifier is not used during its training. A confusion matrix was calculated by comparing the true label of a data point with the predicted label during cross-validation. The whole process was repeated 100 times to account for differences in trial selection. The average decoding performance was defined as the average percent correct predictions of the classifier over the repetitions. To compare the decoding performance to chance level, we calculated the average percent of correct predictions using shuffled prediction labels. We defined decoding performance to be significantly above chance level, if the average decoding performance was above the 95th percentile of performances with shuffled labels.

Captions

Figure 1. Spatial delayed-response task and behavioral performance

(A) Behavioral task. The crow moved its head into a light barrier centrally in front of the screen in the go period to start a trial. In the following cue period (300ms) two out of eight gray frames displayed the possible target locations. The presented possible target locations were horizontally aligned. The cue period was followed by the planning period. Here, a rule cue was additionally presented in the center of the screen. The rule cue instructed either the left or right target location for the later response. The planning period length was variable between 1s and 3s. During the planning period, both possible target locations and the rule cue were presented continuously, making a memorization unnecessary. Once the central rule cue vanished, the bird had to rapidly perform a movement to the instructed location within 800ms. Variable planning period length and short response window made a planning of movement beforehand necessary. (B) Left: Rule cues for left and right differed in either their shape or color. Right: All possible target locations. (C) Average behavioral performance for left and right side instructed and different cue types for bird W (black bars) and bird V (hatched bars). Error bars indicate standard error of the mean (SEM).

Figure 2. Neuronal responses to target locations during planning period

(A-B) Example neurons with selective activity for target side and location. The first three panels show a dot-raster histograms (top) with corresponding spike-density functions (bottom) aligned to begin of the planning period. In dot-raster histograms, each dot represents an action potential, and each line represents a trial. Spike-density functions show the average firing rate smoothed with a 300ms Gaussian kernel. Solid vertical lines represent begin of the planning period and dashed vertical lines the begin of the cue period. First panel shows trials sorted by the instructed side. Second panel shows trials sorted for all different rule cues (cf. Figure 1B). Third panel shows trials sorted for all eight different instructed target locations. The polar plot on the right displays the firing rate for all eight target positions averaged over the gray shaded area in the left panels. The arrow represents the preferred direction of the neuron. (A) Example neuron showing preference equal for all target locations on the left side. (B) Example neuron showing preference for target positions on the right side, and additional preference for target locations 2 and 3 within the right side. (C) Polar histogram of preferred directions of all neurons selective in the planning period (n=117). More neurons preferred target locations on the right respectively contralateral side ($p<0.05$).

Figure 3. Neuronal responses during ambiguous trials

(A) Example neuron activity during normal and ambiguous trials. Left panel displays spike-density functions, that are the average firing rates smoothed with a 300ms Gaussian kernel. Solid lines represent neuronal activity during normal trials, and dashed lines activity during ambiguous trials. Blue color indicates later movement to the right, and red colors later movement to the left side target location. The solid vertical line represents begin of the planning period, and the dashed vertical line the begin of the cue period. During normal trials the neuron has a higher firing rate for trials with target locations on the right side. In ambiguous trials the firing rate resembles the firing rate of normal trials with the same target side. Polar plot on the right side, shows the average firing rates for all eight target positions averaged over the gray shaded area on the left side. Average firing rates of normal and ambiguous trials with the same later target location are similar. (B-C) Performance of support vector machine (SVM) classifiers trained on normal trials to predict choices in ambiguous trials. Right distribution is the prediction performances of classifiers trained on real data, left distribution the prediction performance of classifiers trained on data with permuted labels. Horizontal solid line represents the average prediction performance, dashed lines represent the 5th and 95th percentile of predictions. Prediction performance was defined significantly above chance level, if average performance was above the 95th percentile of performances acquired with at classifier trained on permuted data (asterisk signifies significant difference). Classifier were trained to predict target side (B) or target location (C). Classifiers were able to predict chosen target side and location in ambiguous trials significantly above chance based on neuronal data in normal trials. (D) AUROC values for side-selective neurons for normal and ambiguous trials. AUROC is a measure for discriminability of two distributions. While values of 0 and 1 describe strong discriminability, a value of 0.5 describes no difference. AUROC values were calculated to represent preference for the preferred side in normal trials with values larger 0.5. When calculating the AUROC value for side selective cells in ambiguous trials (n=35), values were on average lower than for side selective cells in normal trials (n=84) but significantly higher than 0.5 ($p<0.05$). This indicates that neurons preferred the same side during normal and ambiguous trials.

Figure 4. Neuronal responses to target locations during movement execution

(A) Example neuron with selective activity during movement to target locations. Central polar plot shows the average firing rate during movement to the eight target locations. Arrow indicates the preferred direction of the neuron. Around the polar plot each panel shows a dot-raster histogram with corresponding peristimulus time histogram (PSTH). In dot-raster histograms, each dot represents an action potential, and each line represents a trial. Each PSTH is the sum of action potentials in the dot-raster histogram in bins of 50ms. Vertical lines represent the peck on the target location. Shaded area is the analysis window. The example neuron increases its activity selectively only for movement to target positions 7 and 8, with only minor increases to neighboring locations and no firing for all other positions. (B) Polar histogram of preferred directions of all neurons with selective activity during movement execution (n=158). Preferred directions were evenly distributed to various directions.

Figure 5. Comparison of selective activity during planning and response period

(A) Tuning of an example neuron selective during both planning and response period. Polar plots show the average firing rate during planning and response period for all eight target locations. Arrows represent preferred directions. While the neuron was selectively preferring target locations around location 1 both during planning and in the response period, the tuning seems sharper during the response period. (B-C) Measure for tuning sharpness in planning and response period for (B) neurons selective in both periods and (C) all remaining selective neurons. Von Mises Kappa is a measure for tuning sharpness of circular data with larger values indicating sharper tuning. Horizontal black lines indicate average Kappa. For both neurons selective in (B) both or (C) only one period, tuning was significantly sharper during the response period. (D-E) Cross-validation performance and confusion matrix for support vector machine (SVM) classifier for (D) planning and (E) response period data. Right panel shows average decoding performance, that is the average of correct predictions over all target positions in a five-fold cross-validation. Dashed line represents chance level, that is the 95th percentile of decoding performances using a classifier trained on permuted data. Left panel shows the confusion matrix, that displays for each true label target location the percent predicted labels. (D) Average decoding performance during the planning period was above chance level (right panel). If prediction errors occurred, they were mainly confined to the correct target side, indicating a population encoding of target side and location. Prediction performance was better for target locations on the right side. (E) Average decoding performance during movement execution in the response period, was highly above chance level. If errors occurred, they were to neighboring positions, indicating a population encoding of target location.

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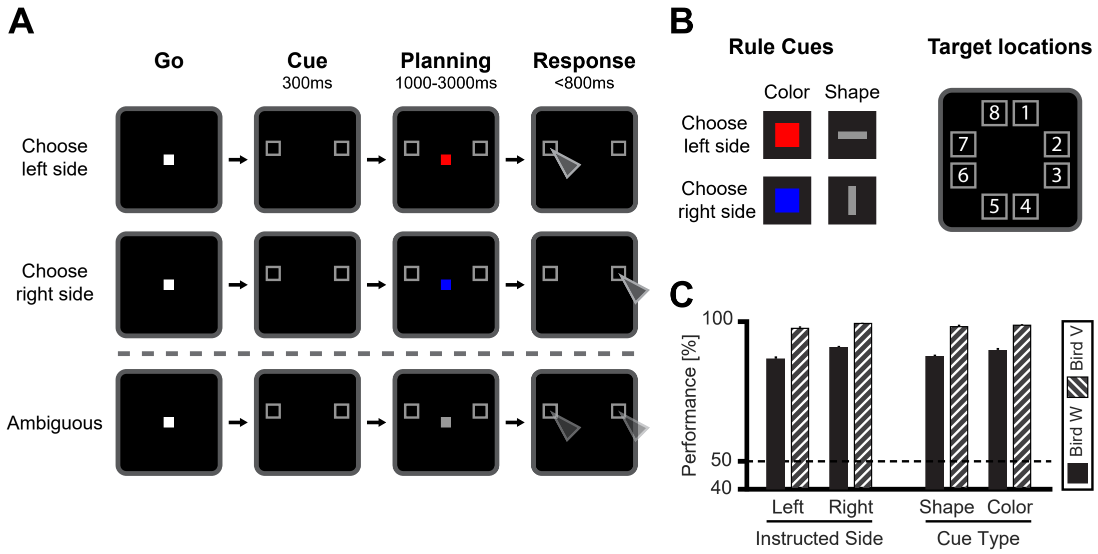
A
Go
Cue
300ms
Planning
1000-3000ms
Response
<800ms
Choose left side
Choose right side
Ambiguous
B
Rule Cues
Color
Shape
Choose left side
Choose right side
Target locations
8
1
7
2
6
3
5
4
C
Performance [%]
100
50
40
Left
Right
Instructed Side
Shape
Color
Cue Type
Bird W
Bird V

Figure 1

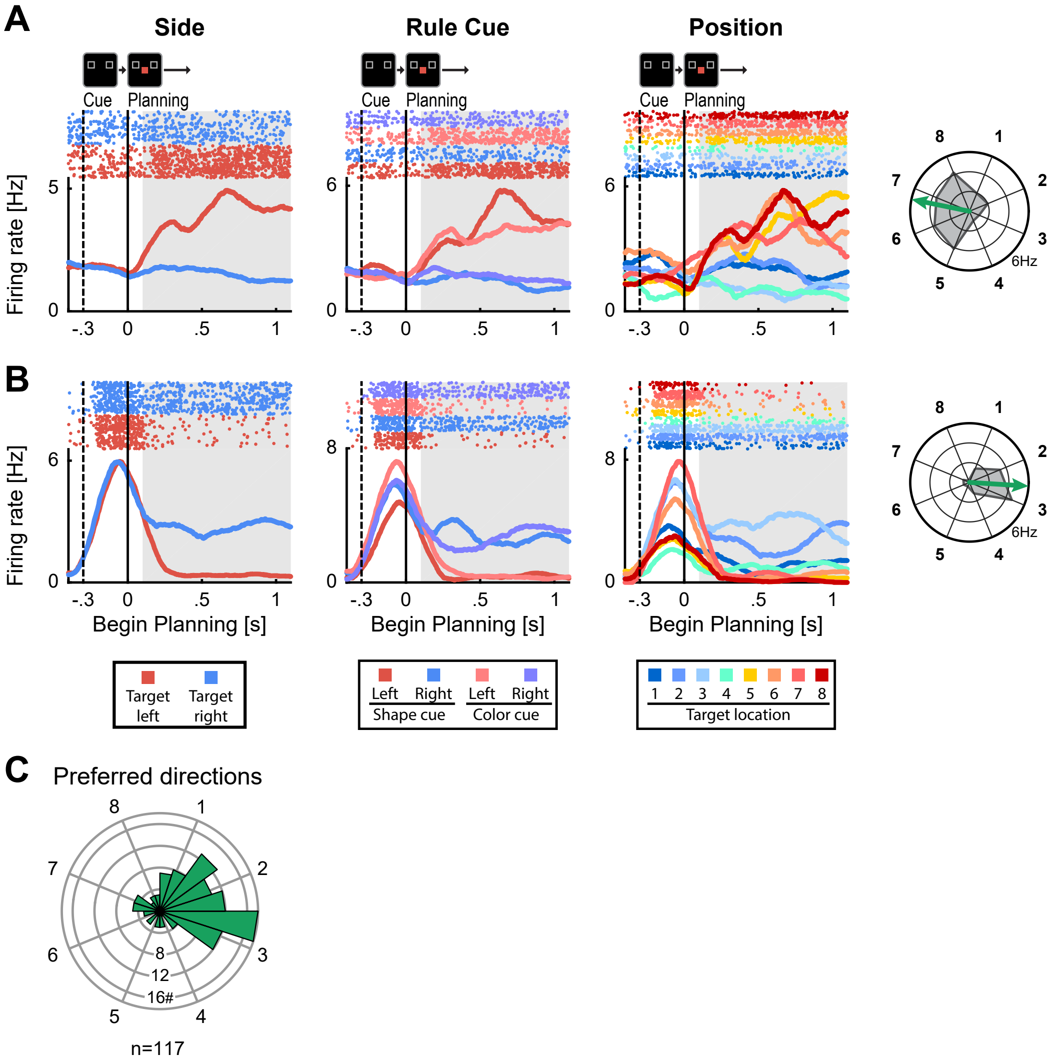
A
Side
Rule Cue
Position
Cue
Planning
Firing rate [Hz]
5
6
0
-.3
0
.5
1
8
1
7
2
6
3
5
4
6Hz
B
Firing rate [Hz]
8
Begin Planning [s]
Target left
Target right
Left
Right
Shape cue
Left
Right
Color cue
1 2 3 4 5 6 7 8
Target location
C
Preferred directions
12
16#
n=117

Figure 2

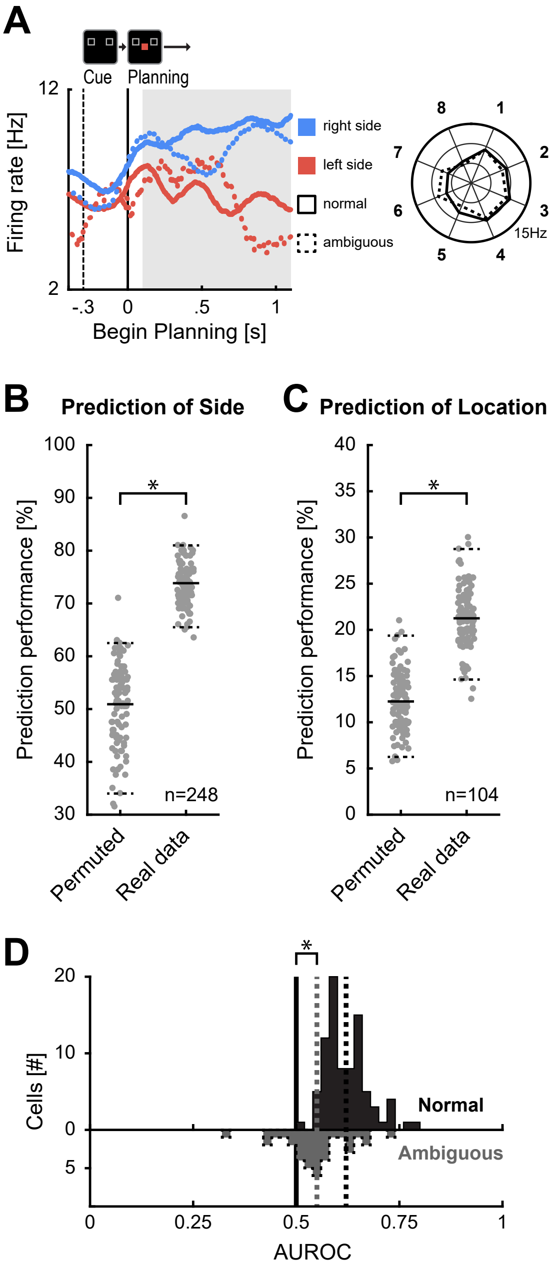
A
Cue
Planning
Firing rate [Hz]
12
2
-.3
0
.5
1
Begin Planning [s]
right side
left side
normal
ambiguous
8
1
2
3
4
5
6
7
15Hz
B
Prediction of Side
Prediction performance [%]
100
90
80
70
60
50
40
30
*
n=248
Permuted
Real data
C
Prediction of Location
Prediction performance [%]
40
35
30
25
20
15
10
5
0
*
n=104
Permuted
Real data
D
Cells [#]
20
10
0
5
*
Normal
Ambiguous
0
0.25
0.5
0.75
1
AUROC

Figure 3

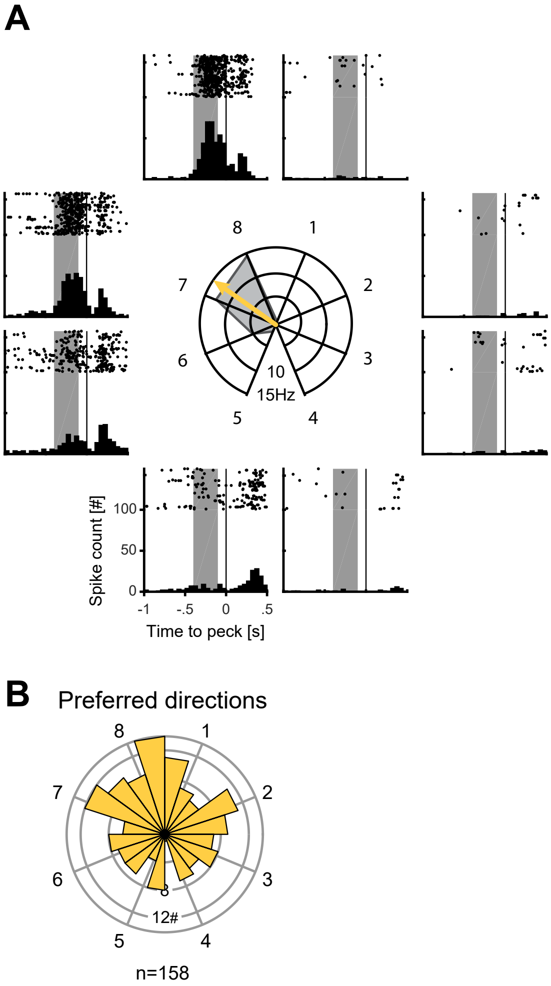
A
8
1
7
2
6
3
10
15Hz
5
4
Spike count [#]
100
50
0
-1
-.5
0
.5
Time to peck [s]
B
Preferred directions
8
1
7
2
6
3
8
12#
5
4
n=158

Figure 4

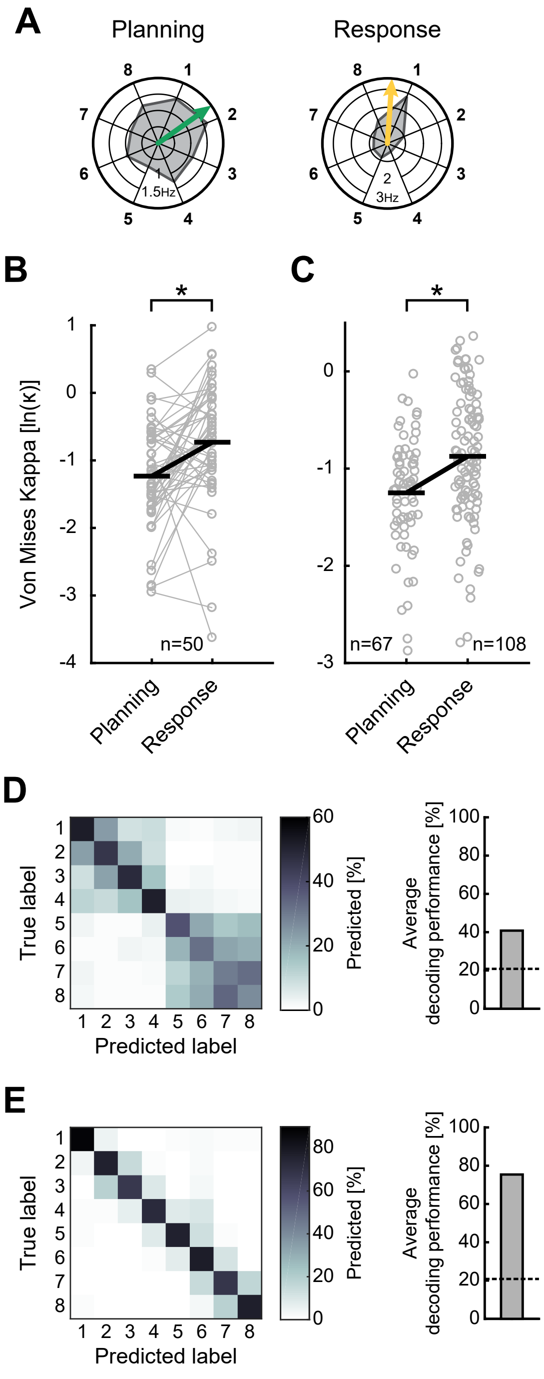
A
Planning
Response
8
1
2
3
4
5
6
7
1
1.5Hz
2
3Hz
B
C
*
Von Mises Kappa [ln(κ)]
1
0
-1
-2
-3
-4
n=50
n=67
n=108
Planning
Response
D
E
True label
Predicted label
Predicted [%]
Average decoding performance [%]
1 2 3 4 5 6 7 8
0
20
40
60
80
100

Figure 5