Acoustic orientation in the dark:

About how the brain processes naturalistic echolocation sequences in the fruit-eating bat *Carollia perspicillata*

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Datum der Dispuation:

“Ought we, for instance, to begin by discussing each separate species - man, lion, ox, and the like - taking each kind in hand independently of the rest, or ought we rather to deal with the attributes which they have in common in virtue of some common element of their nature, and proceed from this as a basis for the consideration of them separately”

Aristotle, ca. 350 BC

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Zusammenfassung

Einführung in die Echoortung und Fragestellung der Arbeit

Fledermäuse können sich akustisch mithilfe der Echoortung in kompletter Dunkelheit orientieren. Dabei stoßen sie Echoortungsrufe aus, die für den Menschen aufgrund der hohen Frequenz nicht hörbar sind. Diese Rufe werden an umliegenden Objekten reflektiert. Die daraus hervorgehenden Echos verwenden Fledermäuse, um ein akustisches Bild ihrer Umgebung zu erzeugen. Zeitliche und spektrale Echoeigenschaften hängen von Position und Art der umliegenden Objekte ab (Wohlgemuth et al. 2016b). Die Informationen eines Echos entsprechen einer Momentaufnahme der Umgebung, ähnlich wie ein Einzelbild eines Films. Fledermäuse stoßen Echoortungsrufe mit einer hohen Wiederholungsrate aus, um eine hohe räumliche Auflösung zu erhalten. Die daraus hervorgehenden Echos werden zeitlich integriert, wodurch eine kontinuierliche Repräsentation der Umgebung entsteht (Simmons 2012; Moss and Surlykke 2001, 2010).

Für Kollisionsfreie Flüge ist eine exakte Berechnung der Distanz zu umliegenden Objekten entscheidend. Fledermäuse ermitteln die Distanz mithilfe der sogenannten Echo-Verzögerung (Kössl et al. 2014; Wohlgemuth et al. 2016b). Die Echo-Verzögerung entspricht der Dauer vom Rufausstoß bis zum Empfang des Echos und hängt von der Distanz ab, die beide Signale zurücklegen müssen. Neurone, die selektiv auf bestimmte Echo-Verzögerungen antworten, sogenannte „*delay-tuned neurons*“, findet man entlang der aufsteigenden Hörbahn ab dem auditorischen Mittelhirn (colliculus inferior = IC; Wenstrup et al. 2012; Macías et al. 2016). In der Regel werden „*delay-tuned neurons*“ mithilfe von künstlichen Stimuli, die nur einen Teil des natürlichen Echoortungsrufes und des Echos widerspiegeln untersucht (Kössl et al. 2014). Dabei werden die Stimuli dem Tier als Ruf-Echo Paare mit randomisierten Echo-Verzögerungen präsentiert. Da Echoortungsrufe jedoch mit hoher Rate ausgestoßen werden, wäre es nicht verwunderlich, wenn die zeitliche Struktur einer Echoortungssequenz entscheidend für die neuronale Verarbeitung ist. Frühere Studien haben gezeigt, dass eine Erhöhung der Stimulusrate bei Neuronen des ICs und des auditorischen Kortex (AC) eine selektivere Reaktion auf unterschiedliche Stimulus-Parameter auslösen (Bartenstein et al. 2014; Galazyuk et al. 2000; Wong et al. 1992; Zhou and Jen 2006; Wu and Jen 1996; Chen and Jen 1994). Allerdings wurden dabei künstliche Echoortungssequenzen als Stimuli verwendet. Daher war das Hauptziel dieser Arbeit die neuronale Verarbeitung natürlicher Echoortungssequenzen im IC und im AC der früchtefressenden Fledermaus *Carollia perspicillata* zu untersuchen. Neben dem vergleichenden Ansatz der neuronalen Verarbeitung zwischen IC und AC untergliedert sich die Arbeit in drei Teilfragen. Im Ersten Teil untersuchte ich, welchen Einfluss der zeitliche Kontext einer natürlichen Echoortungssequenz auf die neuronale Verarbeitung hat. Im Zweiten Teil untersuchte ich, wie Echoortungssequenzen, die Informationen von bis zu drei Objekten beinhalten

neuronal verarbeitet werden. Dabei richtete sich das Hauptaugenmerk darauf, ob die Neurone Echoinformationen aller Objekte oder nur von jeweils einem Objekt verarbeiten. Im dritten Teil meiner Arbeit untersuchte ich den Einfluss einer Beschallung mit Echoortungssignalen auf das Echoortungsverhalten einer Fledermaus. Auch wurde die Robustheit neuronaler Antworten auf eine Echoortungssequenz bei Anwesenheit von weiteren Echoortungssignalen anderer Artgenossen analysiert. Die Ergebnisse des dritten Teils könnten erklären, wie eine Echoortung in Anwesenheit mehrerer Fledermäuse möglich ist.

Schaukel-Paradigma: Tonaufnahmen natürlicher Echoortungssequenzen

Für die Aufnahme natürlicher Echoortungssequenzen wurde die Fledermaus in einer Schaukel positioniert (Henson et al. 1982). Durch Schwingen der Schaukel wurde der Fledermaus eine Flugsequenz von 4 m simuliert. Währenddessen stößt die Fledermaus Echoortungssignale aus, welche an umliegenden Objekten reflektiert werden. Um die Rufe und Echos aufzunehmen wurde ein im Ultraschallbereich empfindliches Mikrofon an der schwingenden Schaukel befestigt. Dabei wurde darauf geachtet, dass der Abstand des Mikrofons zu den Ohren des Tieres maximal 4 cm betrug. Durch Positionieren verschiedener Objekte in die Flugbahn der Schaukel konnten Echoquellen manuell bestimmt werden.

Mithilfe dieses Paradigmas wurden in dieser Arbeit zwei unterschiedliche Stimulus-Szenarien simuliert. Für die Aufnahme einer „*simplen*“ Echoortungssequenz wurde eine Plexiglasplatte so positioniert, dass die Fledermaus am Ende der Vorwärtsschwingung etwa 30 cm vor der Platte zum Stehen kam. Die während der Vorwärtsschwingung aufgenommene Echoortungssequenz entsprach einem Stimulus-Szenario, das die Fledermaus erfahren würde, wenn sie ein statisches Objekt anfliegt. Bei der „*multiple-object*“ Echoortungssequenz waren zwei weitere Objekte vorhanden. Objekt A, ein Fels aus Pappmaché, war vom Startpunkt der Schwingung 2,70 m entfernt und wurde von der Schaukel überschwungen, was der Fledermaus ein Überfliegen eines Objekts simuliert. In einer Entfernung von 1,30 m hinter Objekt A befand sich Objekt B, eine quadratische Holzplatte in einer Höhe von 1,10 m. Objekt B wurde so positioniert, dass die schwingende Schaukel mit der Fledermaus 10 cm davor zum Stillstand kam. 20 cm hinter Objekt B befand sich Objekt C, die Plexiglasplatte aus der „*simplen*“ Echoortungssequenz. Durch das Positionieren der drei Objekte in unterschiedlichen Distanzen folgten jedem Echoortungsruf mindestens zwei objektspezifische Echos. Die physikalischen Rufparameter der in der Schaukel aufgenommenen Echoortungssequenzen entsprachen den Signalparametern von freifliegenden Fledermäusen der Art *C. perspicillata* (Thies et al. 1998).

Echoortungssequenzen evozieren neuronale Suppression, die die Selektivität der neuronalen Antwort auf bestimmte Ruf-Echo Paare erhöht! (Beetz et al. 2016b; Beetz et al. submitted A)

Rasch aufeinanderfolgende Stimuli evozieren in der Regel eine neuronale Suppression, wodurch die Neurone schwach bis gar nicht auf nachfolgende Stimuli reagieren (Wehr and Zador 2005; Brosch and Schreiner 1997; Kilgard and Merzenich 1998; Calford and Semple 1995). Die Rufrate von *C. perspicillata* liegt im Bereich zwischen 10 und 40 Hz (Thies et al. 1998). Unter Berücksichtigung von Echos sind akustische Raten zwischen 20 und 80 Hz möglich. Um den Einfluss der akustischen Rate einer natürlichen Echoortungssequenz auf die neuronale Verarbeitung zu testen, wurde die „*simple*“ Echoortungssequenz in ihre einzelnen Ruf-Echo Paare zerlegt. Diese Ruf-Echo Paare wurden einer anästhesierten Fledermaus randomisiert mit einer akustischen Rate von 2.5 Hz vorgespielt („*element situation*“), während die neuronale Aktivität von IC oder AC aufgenommen wurde. Zusätzlich wurde die Fledermaus mit der natürlichen Echoortungssequenz stimuliert („*sequence situation*“). Durch Vergleichen der neuronalen Antworten in der „*element*“ und „*sequence situation*“ wurde gezeigt, dass Neurone beider Hirnareale während der „*sequence situation*“ supprimiert wurden. Trotz Suppression konnten Neurone im IC auf jedes akustische Signal in der Echoortungssequenz antworten und die Aktionspotentiale waren zeitlich zum Stimulus synchronisiert. Die Suppression war für die zeitliche Verarbeitung der Echoortungssequenz förderlich, da sie die Rate zwischen dem Signal und dem Rauschen („*signal-to-noise ratio*“) signifikant erhöht hat. Außerdem reagierten Neurone des ICs in der „*sequence situation*“ selektiver auf bestimmte Ruf-Echo Paare als in der „*element situation*“.

Im AC ist die Suppression ebenfalls für die zeitliche Verarbeitung der Echoortungssequenz förderlich. Die kortikalen Neurone antworteten in der „*sequence situation*“ ebenfalls selektiver auf bestimmte Ruf-Echo Paare als in der „*element situation*“. Während der „*sequence situation*“ erholten sich die kortikalen Neurone teilweise von der Suppression und antworteten selektiv auf bestimmte Ruf-Echo Paare. „*Delay-tuned neurons*“ sind im Kortex entlang der rostro-caudalen Achse topografisch angeordnet (Chronotopie; Hagemann et al. 2010). Mithilfe von selbstgebauten Glas-Multielektroden war ich in der Lage, von bis zu acht Neuronen zeitgleich abzuleiten. So konnte ich die Organisation der Chronotopie charakterisieren während die Fledermaus mit natürlichen Echoortungssequenzen stimuliert wurde. Die durch die „*sequence situation*“ evozierte Suppression und die daraus folgende selektive neuronale Antwort auf bestimmte Ruf-Echo Paare führten dazu, dass die Chrontopie eindeutiger war als in der „*element situation*“.

Mittelhirnneurone können Echos mehrerer Objekte parallel verarbeiten, wohingegen kortikale Neurone leidglich Echos vom nächstgelegenen Objekt verarbeiten! (Beetz et al. 2016a; Beetz et al. submitted A)

Die natürliche Umgebung von Fledermäusen besteht in der Regel nicht nur aus einem Objekt. Echoortungsrufe werden von mehreren umliegenden Objekten reflektiert, wodurch mehrere Echos einem Ruf folgen (Moss and Surlykke 2010). In dieser Arbeit habe ich die neuronale Verarbeitung unter solchen Bedingungen getestet, indem ich anästhesierte Fledermäuse mit der „*multiple-object*“ Echoortungssequenz beschallt habe. Um herauszufinden, welches Objekt die neuronale Antwort auf diese Sequenz am Stärksten beeinflusst, wurde die „*multiple-object*“ Echoortungssequenz in drei „*single-object*“ Echoortungssequenzen umgewandelt. Hierfür wurden manuell die Echos von zwei Objekten gelöscht. Beispielsweise wurde durch das Löschen der Echos von Objekt B und Objekt C eine „*single-object*“ Echoortungssequenz erstellt, die nur Echos von Objekt A beinhaltete. Drei „*single-object*“ Echoortungssequenzen und die „*multiple-object*“ Echoortungssequenz wurden dem Tier präsentiert und die entsprechenden neuronalen Antwortmuster miteinander verglichen.

Da Neurone im IC relativ schwach supprimiert werden, reagierten sie auf alle Echos der „*multi-object*“ Echoortungssequenz. Allerdings fiel auf, dass in etwa 72% der Neurone Objekt B den stärksten Einfluss auf die neuronale Antwort hatte. Dieses Ergebnis war teilwiese darauf zurückzuführen, dass die meisten Echos der „*multiple-object*“ Echoortungssequenz von Objekt B stammten. Im AC dagegen reagierten die Neurone aufgrund der starken Suppression ausschließlich auf Echos des nächstgelegenen Objekts. Bei der Aufnahme der „*multiple-object*“ Echoortungssequenz hat die schaukelnde Fledermaus Objekt A nach etwa der Hälfte der Schwingung passiert, sodass nur noch Echos von Objekt B und Objekt C in der restlichen Sequenz repräsentiert waren. Dadurch war ab der zweiten Hälfte der „*multiple-object*“ Echoortungssequenz Objekt B das nächstgelegene Objekt zum Tier. In der ersten Hälfte der „*multiple-object*“ Echoortungssequenz reagierten Neurone im IC und AC verstärkt auf Objekt A, wohingegen sie in der zweiten Hälfte der Sequenz verstärkt auf Objekt B reagierten. Zusammenfassend verhindert die neuronale Suppression, dass Echos aller Objekte gleichermaßen verarbeitet werden. Verhaltensbiologisch ist die übergeordnete Verarbeitung des nächstgelegene Objekts wichtig, um Kollisionen zu vermeiden.

In geräuschvoller Umgebung erhöht *C. perspicillata* die Rufrate, um eigene Echoortungssignale neuronal besser verarbeiten zu können! (Beetz et al. submitted B)

Mithilfe der Echoortung können sich Tiere unabhängig von äußeren Umwelteinflüssen, wie zum Beispiel Lichtverhältnissen, orientieren. Allerdings müssen sie ihre eigenen Echoortungssignale von denen ihrer Artgenossen unterscheiden. Jedes akustische Signal kann potentiell mit den eigenen Echoortungssignalen der Fledermaus interferieren. In der Vergangenheit wurden einige Verhaltensanpassungen als Antwort einer solchen Situation beschrieben (siehe Übersichtsartikel von; Ulanovsky and Moss 2008). Allerdings wurde neurophysiologisch bisher noch nicht getestet, ob diese Anpassungen die neuronale Verarbeitung verhaltensrelevanter Signale erleichtert. Da bisher noch keine Verhaltensanpassungen auf Beschallungen in *C. perspicillata* beschrieben worden sind, untersuchte ich durch Beschallungsexperimente in einem Flugraum, ob und wie *C. perspicillata* das Echoortungsverhalten anpasst. Während der Beschallung stößte *C. perspicillata* ihre Rufe verstärkt in Gruppen aus. Dabei verringerte sie die Rufpausen innerhalb der Gruppe, wodurch die Rufgruppen zeitlich konzentrierter waren als die Rufgruppen, die ohne Beschallung ausgestoßen worden sind. Die erhöhte Rufrate innerhalb einer Gruppe scheint sinnvoll, da sie die räumliche Auflösung der empfangenen Echos erhöhen würde. Dadurch könnte der Wegfall einiger Echoinformationen durch akustische Interferenzen kompensiert werden. Unter Berücksichtigung der Ergebnisse der vorangegangenen neurophysiologischen Experimente ist es fraglich, ob eine Erhöhung der Rufrate die neuronale Verarbeitung vereinfacht. Schließlich könnte eine Erhöhung der Rufrate auch eine Verstärkung der neuronalen Suppression nach sich ziehen. Daher wurde der Einfluss der akustischen Interferenzen (Maskierer) in Form von Echoortunssignalen anderer Tiere auf die neuronale Verarbeitung der „*simplen*“ Echoortungssequenz untersucht. Trotz Maskierer konnten Neurone im IC auf die „*simple*“ Echoortungssequenz antworten. Im Vergleich dazu bewirkten die Maskierer im AC eine Erhöhung der neuronalen Suppression, wodurch die neuronale Selektivität ebenfalls erhöht wurde. Dadurch waren die Neurone weniger anfällig auf Interferenzen und konnten Informationen der „*simplen*“ Echoortungssequenz verarbeiten. Zusammenfassend wäre eine Erhöhung der Rufrate auf Antwort von akustischen Interferenzen eine geeignete Anpassung, um eine neuronale Verarbeitung einer „*simplen*“ Echoortungssequenz zu sichern.

Schlussfolgerung

Die Ergebnisse der vorliegenden Arbeit zeigen, dass die natürliche zeitliche Struktur der Stimuli die neuronale Verarbeitung entscheidend bestimmt. Die natürlichen Echoortungssequenzen bewirkten eine neuronale Suppression, die sich vom IC zum AC erhöht. In beiden Hirnarealen erhöht die Suppression die neuronale Selektivität auf bestimmte Ruf-Echo Paare der Echoortungssequenz. Im Gegensatz zum AC wird im IC die neuronale Antwort auf die akustischen Signale der Echoortungssequenz nicht völlig supprimiert, sodass die Aktionspotentiale im IC zeitlich an dem Stimulus synchronisiert sind. Der IC kann so den zeitlichen Verlauf von Echoortungssequenzen detailliert repräsentieren. Die zeitliche Verarbeitung wird auch bei höheren akustischen Raten, wie durch das Hinzufügen von Echoinformationen mehrere Objekte oder durch Maskierer nicht negativ beeinflusst. Im Gegensatz zum IC ist die Suppression im AC stärker. Die Suppression agiert auch auf kortikaler Ebene als Selektionsfilter und bewirkt, dass Neurone während der Stimulation mit einer Echoortungssequenz massiv supprimiert werden. Nur in Antwort auf bestimmte Ruf-Echo Paare erholen sich die Neurone von der Suppression und können diese so selektiv verarbeiten. Die starke Suppression bewirkt außerdem, dass primär Echos vom nächstgelegenen Objekt verarbeitet werden.

Zusammenfassend zeigen die Ergebnisse dieser Arbeit, dass die zeitliche Verarbeitung von rasch aufeinanderfolgenden Stimuli nicht zwingend durch das Einwirken der neuronalen Suppression verschlechtert wird. Die Suppression agiert vielmehr als Filter und gewährleistet eine selektive Verarbeitung bestimmter sensorischer Informationen innerhalb einer Sequenz.

Ebenso zeigen die Ergebnisse dieser Arbeit, dass nicht nur natürliche Stimuli in zukünftigen neurophysiologischen Studien berücksichtig werden sollten, sondern diese auch in einem natürlichen zeitlichen Kontext dem zu untersuchenden Tier präsentiert werden sollten. Unter natürlichen Verhaltensbedingungen verarbeiten sensorische Systeme in der Regel keine zeitlich voneinander isolierten Stimuli, sondern vielmehr eine Abfolge von Stimuli.

Summary

Echolocation allows bats to orientate in darkness without using visual information. Bats emit spatially directed high frequency calls and infer spatial information from echoes coming from call reflections in objects (Simmons 2012; Moss and Surlykke 2001, 2010). The echoes provide momentary snapshots, which have to be integrated to create an acoustic image of the surroundings. The spatial resolution of the computed image increases with the quantity of received echoes. Thus, a high call rate is required for a detailed representation of the surroundings.

One important parameter that the bats extract from the echoes is an object's distance. The distance is inferred from the echo delay, which represents the duration between call emission and echo arrival (Kössl et al. 2014). The echo delay decreases with decreasing distance and delay-tuned neurons have been characterized in the ascending auditory pathway, which runs from the inferior colliculus (Wenstrup et al. 2012; Macías et al. 2016; Wenstrup and Portfors 2011; Dear and Suga 1995) to the auditory cortex (Hagemann et al. 2010; Suga and O'Neill 1979; O'Neill and Suga 1982).

Electrophysiological studies usually characterize neuronal processing by using artificial and simplified versions of the echolocation signals as stimuli (Hagemann et al. 2010; Hagemann et al. 2011; Hechavarría and Kössl 2014; Hechavarría et al. 2013). The high controllability of artificial stimuli simplifies the inference of the neuronal mechanisms underlying distance processing. But, it remains largely unexplored how the neurons process delay information from echolocation sequences. The main purpose of the thesis is to investigate how natural echolocation sequences are processed in the brain of the bat *Carollia perspicillata*. Bats actively control the sensory information that it gathers during echolocation. This allows experimenters to easily identify and record the acoustic stimuli that are behaviorally relevant for orientation. For recording echolocation sequences, a bat was placed in the mass of a swinging pendulum (Kobler et al. 1985; Beetz et al. 2016b). During the swing the bat emitted echolocation calls that were reflected in surrounding objects. An ultrasound sensitive microphone traveling with the bat and positioned above the bat's head recorded the echolocation sequence. The echolocation sequence carried delay information of an approach flight and was used as stimulus for neuronal recordings from the auditory cortex and inferior colliculus of the bats.

Presentation of high stimulus rates to other species, such as rats, guinea pigs, suppresses cortical neuron activity (Wehr and Zador 2005; Creutzfeldt et al. 1980). Therefore, I tested if neurons of bats are suppressed when they are stimulated with high acoustic rates represented in echolocation sequences (sequence situation). Additionally, the bats were stimulated with randomized call echo elements of the sequence and an interstimulus time interval of 400 ms (element situation). To quantify neuronal suppression induced by the sequence, I compared the response pattern to the sequence situation with the

concatenated response patterns to the element situation. Surprisingly, although the bats should be adapted for processing high acoustic rates, their cortical neurons are vastly suppressed in the sequence situation (Beetz et al. 2016b). However, instead of being completely suppressed during the sequence situation, the neurons partially recover from suppression at a unit specific call echo element. Multi-electrode recordings from the cortex allow assessment of the representation of echo delays along the cortical surface. At the cortical level, delay-tuned neurons are topographically organized. Cortical suppression improves sharpness of neuronal tuning and decreases the blurriness of the topographic map. With neuronal recordings from the inferior colliculus, I tested whether the echolocation sequence also induced neuronal suppression at subcortical level. The sequence induced suppression was weaker in the inferior colliculus than in the cortex. The collicular response makes the neurons able to track the acoustic events in the echolocation sequence. Collicular suppression mainly improves the signal-to-noise ratio. In conclusion, the results demonstrate that cortical suppression is not necessarily a shortcoming for temporal processing of rapidly occurring stimuli as it has previously been interpreted.

Natural environments are usually composed of multiple objects. Thus, each echolocation call reflects off multiple objects resulting in multiple echoes following the calls. At present, it is largely unexplored how neurons process echolocation sequences containing echo information from more than one object (multi-object sequences). Therefore, I stimulated bats with a multi-object sequence which contained echo information from three objects. The objects were different distances away from each other. I tested the influence of each object on the neuronal tuning by stimulating the bats with different sequences created from filtering object specific echoes from the multi-object sequence. The cortex most reliably processes echo information from the nearest object whereas echo information from distant objects is not processed due to neuronal suppression. Collicular neurons process less selectively echo information from certain objects and respond to each echo.

For proper echolocation, bats have to distinguish between own biosonar signals and the signals coming from conspecifics. This can be quite challenging when many bats echolocate adjacent to each other. In behavioral experiments, the echolocation performance of *C. perspicillata* was tested in the presence of potentially interfering sounds. In the presence of acoustic noise, the bats increase the sensory acquisition rate which may increase the update rate of sensory processing. Neuronal recordings from the auditory cortex and inferior colliculus could strengthen the hypothesis. Although there were signs of acoustic interference or jamming at neuronal level, the neurons were not completely suppressed and responded to the rest of the echolocation sequence.

Introduction

Spatial orientation with special emphasis on orientation under dark light conditions

Spatial orientation is fundamental to and tightly interconnected with locomotion. It involves many neuronal computations (Figure 1). To plan and choose the direction of movement, animals have to know their position in relation to the goal (egocentric representation (Sarel et al. 2017)) and the goal's position in relation to other orientation cues (allocentric representation (Ekstrom et al. 2014)). After deciding a steering direction, the animal's motor command system elicits a goal directed movement. During locomotion, the egocentric representation gets updated which allows adjustments of the steering direction. To understand the closed loop of goal directed locomotion, it is necessary to investigate orientation at multiple organization levels.

During orientation, animals use orientation cues that could range from visual landmarks (Braithwaite and Guilford 1991; Tinbergen and Kruyt 1938; Wehner et al. 1996; Kamil and Jones 1997; Cain and Malwal 2002; Collett et al. 1986; Reese 1989; Tsoar et al. 2011) to compass-like orientation cues like the earth's magnetic field or the polarization pattern of the sky (Homberg et al. 2004; Beetz et al. 2016c; Freas et al. 2017; Dacke et al. 2003; Muheim et al. 2006; Wiltschko and Wiltschko 2005). Animals often rely on several, synergistically working sensory modalities during orientation (Schumacher et al. 2016; Schumacher et al. 2017; Höller and Schmidt 1996). Depending on the availability of orientation cues, animals adapt their orientation strategies. For animals, like bats that live in low light conditions the use of visual cues are limited. Therefore, the animals either have a high visual sensitivity (Dacke et al. 2011; Thomas et al. 2002; Honkanen et al. 2017) or they rely more on other sensory modalities than vision (Catania 2012; von der Emde and Schwarz 2002).

Most bats use their highly sensitive auditory system for short range orientation, with the exception of the mostly visually guided species from the family *Pteropodidae*. The rest of the thesis, unless explicitly stated, focuses on bats that primarily use audition for short range orientation. The following section summarizes the orientation behavior of bats and describes the primary sensory modalities utilized during orientation.

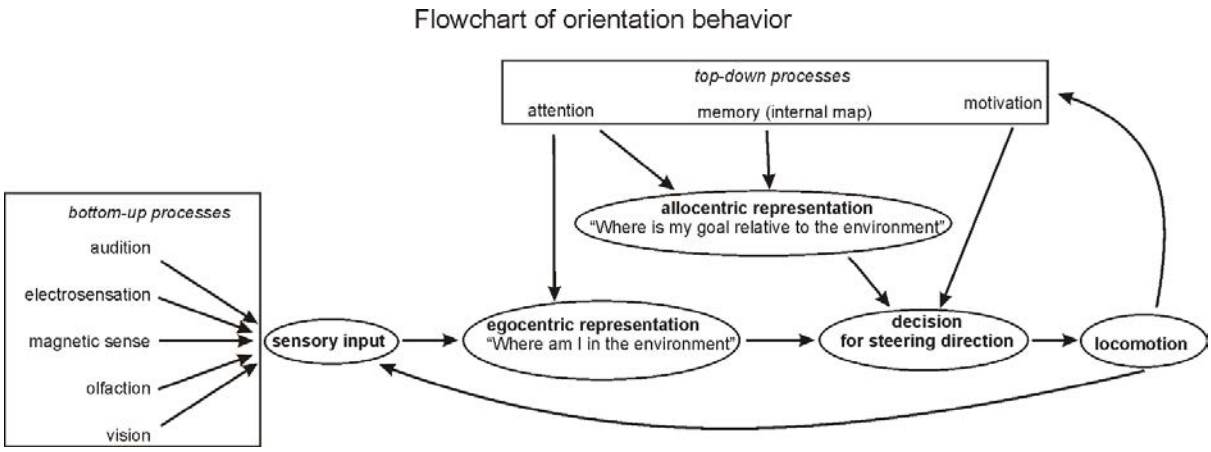

Figure 1 Hypothetical flowchart of orientation behavior. Sensory input (bottom-up processes) provides information to compute the animal's position in relation to the environment (egocentric representation). Top-down processes, like memory, are required to know the goal's localization in relation to the environment (allocentric representation). For both representations the animal's attention is necessary. Egocentric and allocentric representations are compared to determine a steering direction. The motivation determines the threshold for triggering goal-directed locomotion. During locomotion the animal's internal state and the sensory input gets updated which closes the feedback loop of orientation behavior.

Spatial orientation in bats: A behavior based on multimodal integration?

In bats, orientation tasks are highly diverse and can be roughly classified into long, and short to medium range orientation behavior (Figure 2). In long range orientation, bats cover distances of more than a kilometer. Some bats, including *Pteropodidae,* fly several kilometers from their roost towards a feeding place (Williams and Williams 1970; Tsoar et al. 2011; Holland et al. 2006) and some species even migrate seasonally by covering distances of few hundred to thousand kilometers (Davis 1966; Cockrum 1956; Davis and Hitchcock 1965; Cryan 2003; Dietz et al. 2007). When released several kilometers away from the roost, bats can still orientate and find their way back to their roost (Smith and Goodpaster 1958; Williams et al. 1966; Hassell and Harvey 1965; Tsoar et al. 2011; Lindecke et al. 2015). Flight paths of blindfolded bats are tortuous in comparison to the straight flight paths of non-blindfolded bats (Williams and Williams 1970, 1967). Thus, vision seems to be important for keeping a straight flight direction, but it is not ultimately needed for successful homing. Whether bats use visual landmarks, a compass sense, or both during their homing behavior remains obscure. Behavioral studies show that bats likely sense the earth's electromagnetic field, an ability which could be utilized for a magnetic compass (Holland et al. 2006; Holland et al. 2008; Wang et al. 2007b; Dietz et al. 2007). Magnetic compasses have been demonstrated in different migrating animals including birds (Wiltschko and Wiltschko 1972), turtles (Lohmann et al. 2004), fishes (Quinn and Brannon 1982; Walker et al. 1997), and monarch butterflies (Guerra et al. 2014; Etheredge et al. 1999). Surprisingly, the bat's magnetic compass seems to be calibrated by sunset cues (Holland et al. 2010). Whether compass calibration also occurs during the night

by star-, moonlight, or polarized light (Greif et al. 2014; Lindecke et al. 2015) is still under discussion. However, behavioral experiments revealed that bats can see simulated starlight in the lab (Childs and Buchler 1981). Independent from the strategies that the bats use, vision seems to be crucial for long range orientation.

For short to medium range orientation, bats primarily acoustically orientate (Boonman et al. 2013; Stilz and Schnitzler 2012; Kick 1982; Kalko and Schnitzler 1993; Holderied and von Helversen 2003; Kalko and Schnitzler 1989) by emitting sequences of high frequency calls (Pierce and Griffin 1938; Griffin and Galambos 1941; Galambos and Griffin 1942). The propagating calls are reflected off surrounding objects, thus evoking echoes. Bats use the spectro-temporal echo information to create acoustic images of their surroundings (Simmons 2012; Moss and Surlykke 2010, 2001). This behavior is circumscribed as echolocation behavior and it is common for almost all bats.

Orientation behavior often results from multiple hierarchically organized sensory modalities. In addition to acoustic cues, visual, olfactory, and somatosensory information contribute to hunting and foraging (Boonman et al. 2013; Bell and Fenton 1986; Eklöf et al. 2002; Korine and Kalko 2005; Hessel and Schmidt 1994; Thies et al. 1998; Laska 1990), recognition of conspecifics (Mccracken 1993; Höller and Schmidt 1993), or steering during flight (Hahn 1908) for obstacle avoidance (Orbach and Fenton 2010). Audition, associated with the ability to echolocate, dominates the remaining sensory modalities that the bats use for short range orientation. Orientation based on self-generated auditory signals has the advantage of being independent from abiotic factors like light. Moreover, animals can more accurately compute distance and velocity by audition than by vision (Aytekin et al. 2010; Mélcon et al. 2011). Despite the aforementioned advantages provided by acoustic orientation, echolocation can also be quite challenging. For instance, when many bats echolocate adjacent to each other, echolocation signals from different bats may interfere with each other. The acoustic interference may mask or jam the perception of the bat's individual echolocation signals (Ulanovsky et al. 2004). To prevent or reduce the chance of being jammed under these circumstances, bats profit from different behavioral adaptations that are reviewed later in detail (Suga et al. 1983; Ulanovsky and Moss 2008). Additionally, an internal map of the environment could facilitate orientation in noisy environments. The risk of being jammed increases with signal density and is therefore highest in areas where many bats echolocate. In addition to echolocation information, bats could rely on learned flight paths to orientate in familiar areas (Falk et al. 2014; Kong et al. 2016; Barchi et al. 2013; Neuweiler and Möhres 1967; Knowles et al. 2015). Memorized flight paths are extremely robust and persist even when obstacles are removed (Neuweiler and Möhres 1967; Kong et al. 2016). Field experiments showed that individual bats forage in a constant area on subsequent nights (Neuweiler et al. 1987). Thus, the bats may profit from an internal representation of familiar environments. Spatial memory allows bats to focus their echolocation behavior to the most relevant objects like food or conspecifics (Neuweiler and Möhres 1967; Barchi et al. 2013).

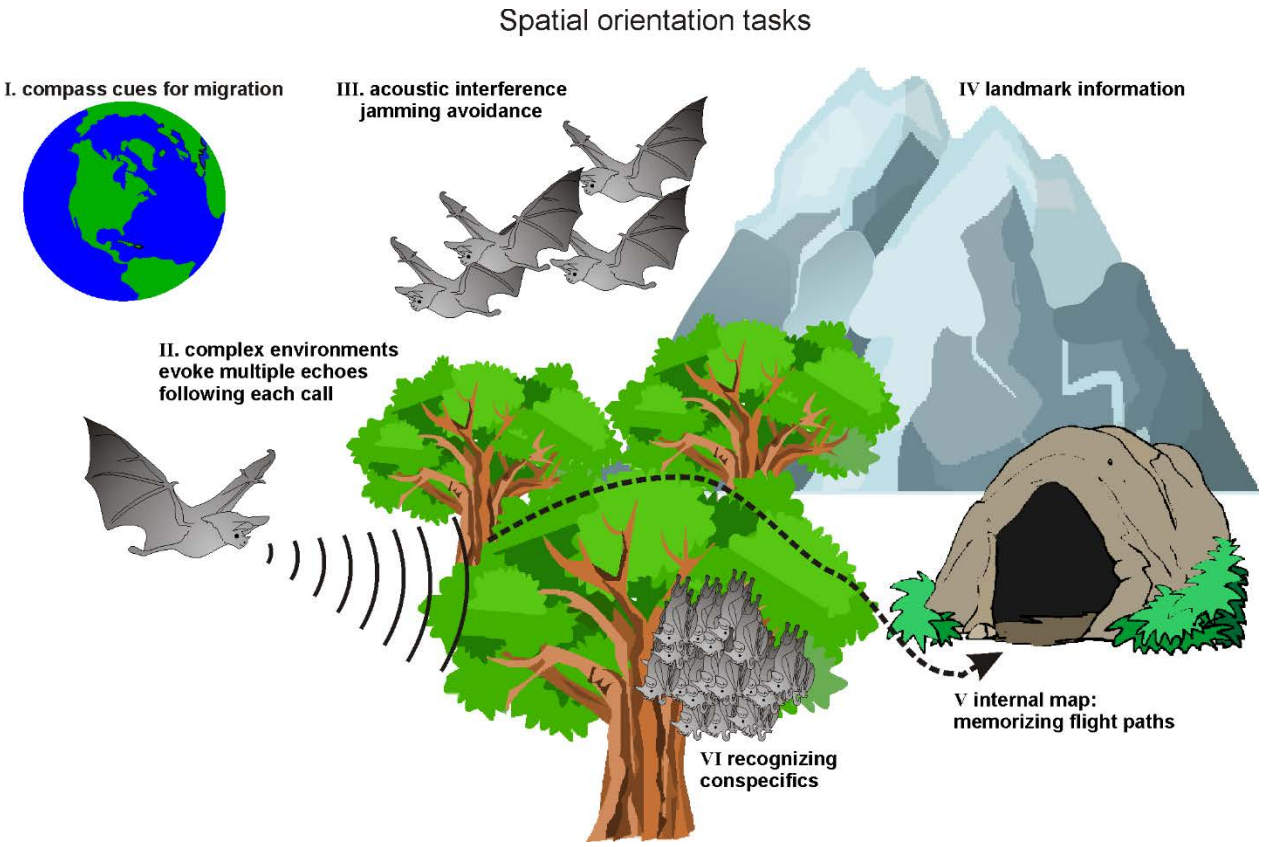

Figure 2 A selection of orientation tasks of bats. (I) Some species of bats migrate seasonally with the help of compass cues. During short to medium range orientation, the bats acoustically orientate with the aid of echolocation. (II) Echolocation is demanding in complex environments where multiple echoes follow each echolocation call. (III) Echolocation signals of conspecifics could acoustically interfere and bats may behaviorally adapt to avoid jamming. (IV) Landmarks and (V) internal maps facilitate orientation in noisy but familiar environments. (VI) In the colony, the bats need to recognize conspecifics including harem members or pubs.

In summary, orientation behavior putatively represents the result of many synergistically working sensory modalities. For successful long range orientation, vision may be important, whereas for short and medium range orientation, the auditory sense is most crucial for the bats. Visual and olfactory cues provide supplementary information for short and medium range orientation. The following chapter describes in more detail the echolocation behavior of bats.

Echolocation behavior

In 1908, Walter Louis Hahn quantitatively showed that bats use their auditory system for flight guidance (Hahn 1908). Hahn was motivated by former experiments on the orientation behavior of bats conducted by the Italian biologist Lazzaro Spallanzani in 1790's. However, Walter Louis Hahn could not explain the nature of the acoustic signals that the bats use for orientation. Until 1920, as Hamilton Hartridge proposed that bats emit high frequency calls, inaudible to humans and that they use the echoes for spatial orientation (Hartridge 1920). Donald R. Griffin experimentally confirmed Hartridge's hypothesis and introduced the term echolocation (Griffin 1944; Pierce and Griffin 1938).

For understanding how echolocation works it is helpful to imagine oneself as a bat. Visualize a scenario where you have to orientate in a completely dark cave. A flashlight helps you to orientate, but, to save power you turn on and off the flashlight from time to time. Only during the brief episodes where the light is turned on you receive an update from the surrounding. During the rest of the time you are completely blind and do not receive any orientation cues. Therefore, you need to extrapolate from the images perceived during the light episodes. With that in mind, you want to move, without crashing into obstacles, at a velocity ranging from 3 to 7 m/s which represents the natural flight speed of a bat (von Busse et al. 2014). This orientation task might be highly demanding for you, but bats succeed daily at similar situations. The light flashes from our experiment correspond to the echoes that the bats perceive. To receive a continuous representation of the environment, we need high repetition rates of switching the flashlight on and off. This would be especially necessary in conditions with many objects. Thus, it is unsurprising that bats increase the call rate when navigating in such complex or unfamiliar environments (Surlykke and Moss 2000; Petrites et al. 2009; Barchi et al. 2013; Falk et al. 2014; Kothari et al. 2014; Sändig et al. 2014; Knowles et al. 2015; Wheeler et al. 2016; Kick 1982; Hiryu et al. 2010; Roverud and Grinnell 1985a). When flying bats approach an object, they usually increase their call rate (Simmons et al. 1978; Griffin 1953). Some bat species reach call rates of more than 200 Hz at the terminal stage of an insect capture (Kalko 1995; Jones and Rayner 1988; Kalko and Schnitzler 1989; Griffin 1953; Schnitzler et al. 1987). This stage is also called "terminal buzz" because the temporally down sampled signal from the audio monitor "sounded like a noisy single-cylinder gasoline engine when it is suddenly accelerated from a slow "put-put-put" to a rasping buzz" (Griffin 1953). In frugivorous bats, no terminal buzz has been recorded and the call rates are rarely above 50 Hz (Brinklov et al. 2009; Thies et al. 1998; Korine and Kalko 2005). Thus, the terminal buzz is interpreted to be an adaptation to hunt for insects. Since a high call rate results in a high accuracy of echolocation, high call rates enable bats to react appropriately to fast, erratic, and evasive flight maneuvers of insects (Geberl et al. 2015).

Since the call rate determines the spatial resolution of orientation we can ask ourselves: Why do bats not consistently emit calls at maximum rates? One reason is that call emission is energetically highly

demanding (Speakman et al. 1989). Thus, bats cannot withstand maximum call rates for a long time because of energy limitations. To save energy, bats usually couple their wing beat cycle with the call emission (Suthers et al. 1972; Thies et al. 1998). The second and likely more important reason bats vary call rates is to avoid a call-echo overlap (Schnitzler et al. 1987; Schnitzler 1968) which would complicate to disentangle call and echo information (Altringham 2011; Kalko and Schnitzler 1993; Cahlander et al. 1964). To minimize the risk of call-echo overlaps, echolocation calls are brief and no consecutive call is emitted while the echo from the preceding call is detected (Neuweiler 1990; Dietz et al. 2007; Altringham 2011). In other words, the closer the object is, the faster the echo arrives and the earlier can the bat emit the next call. Thus, object distance restricts the minimum call interval, as well as call duration. To minimize the risk of call echo overlap, bats usually shorten their calls while approaching an object (Holderied et al. 2006; Simmons et al. 1978; Kalko 1995; Kalko and Schnitzler 1989).

Some studies reported that bats often emit grouped calls (Grinnell and Griffin 1958; Galambos and Griffin 1942; Brinklov et al. 2011; Amichai et al. 2015; Roverud and Grinnell 1985b, a; Kothari et al. 2014; Wheeler et al. 2016; Wohlgemuth et al. 2016a; Brinklov et al. 2009) and the tendency of grouping calls increases with the complexity and the unfamiliarity of the environment (Kothari et al. 2014; Petrites et al. 2009; Falk et al. 2014; Sändig et al. 2014; Surlykke et al. 2009a; Fawcett et al. 2015; Moss et al. 2006). Moreover, frugivorous bats increase their rates of call grouping when approaching a ripe fruit hanging from the ceiling (Korine and Kalko 2005). Emitting groups of calls increases the sensory acquisition rate and thus, the spatial resolution for a brief time period.

After describing the temporal properties of the calls and the echolocation sequences, we want to take a closer look at the call spectra. Echolocation calls are usually composed of a downward frequency modulation (FM) or a combination of frequency modulation and constant frequency components (CF) (Neuweiler 1990; Altringham 2011). Bat species exclusively using FM components belong to the group of FM-bats. Species using a combination of CF and FM components are called CF-FM bats. The FM is brief, usually few milliseconds in duration and covers a broad frequency range (Simmons and Young 2010). In the vast majority of bat species, the FM signal is downward frequency modulated, meaning that there is a gradual decrease in frequency from the start to the end of the signal (Altringham 2011). In contrast to the FM signal, the CF signal is a narrowband signal that lasts longer than the FM signals. In CF-FM bats the single components are combined in different ways. In most cases the CF component precedes the FM. Some bat species, like the ones belonging to the family of *Rhinolophidae* have calls consisting of an upward FM followed by a CF and downward FM component (Neuweiler 1990). Fluttering wings of insects create amplitude and frequency modulations in the CF component of the echo (Neuweiler 1990) and helps the bats to detect insects. Objects that move in relation to a bat's own movement induce an upward frequency shift in the echo because of Doppler effects (Goldman and Henson 1977; Suga 1994; Suga et al. 1983). The relatively long lasting CF component allows the bat to detect that frequency shift.

In contrast, a brief and broadband signal like the FM signal is less suited for detection of Doppler effects but is perfectly suited to analyze an object's position, structure, and shape (Simmons 1973; Simmons et al. 1979; Siemers and Schnitzler 2004; Simmons and Stein 1980; Faure and Barclay 1994; Jensen and Miller 1999; Surlykke et al. 1993).

Depending on the bat species, the main energy of the echolocation calls lies in the frequency range between 12-200 kHz (Neuweiler 1990). Most species emit calls in the ultrasonic range (> 20 kHz). There are several reasons for concentrating so much energy into high frequencies. First, as described earlier, echolocation is prone to acoustic interference. Any acoustic signal overlapping the frequency range of the echolocation signals could jam the bats. However, because signal energy of most environmental sounds is concentrated at low frequencies, concentrating the main energy of the calls to high frequencies reduces the risk of being jammed (Altringham 2011). Additionally, high frequency signals are spatially directed and their short wavelengths enable reflections from small objects (Boonman et al. 2013). Although low frequencies travel further than high frequencies, they bypass small targets without being reflected. Thus, small objects like insects can only be detected with high frequency signals (Altringham 2011). High frequencies suffer strongly from atmospheric attenuation which limits the working range of echolocation to a few meters (Kick 1982; Grinnell and Griffin 1958; Lawrence and Simmons 1982).

Many factors determine the spectro-temporal profile of an echolocation call. The call structure varies between bat species (interspecific differences) and correlates with habitat preferences (Dietz et al. 2007; Neuweiler 1990; Griffin and Novick 1955; Aldridge and Rautenbach 1987; Neuweiler 1989; Jones 1999; Schnitzler and Kalko 2001; Fenton and Bell 1981). Individual specific call structures within a bat species (intraspecific differences) (Kalko and Schnitzler 1993; Habersetzer 1981; Thomas et al. 1987) could help individuals for recognizing their own biosonar signals (Obrist 1995; Jones et al. 1991). Depending on the task and the physical structure of the surroundings, bats flexibly adjust their call design to optimize echolocation performance (Simmons et al. 1978; Simmons et al. 1979; Ibanez et al. 2004). Thus, a call emitted in a cluttered environment often structurally differs from a call emitted in an open space (Kalko and Schnitzler 1993; Fenton 1986; Falk et al. 2014; Schnitzler and Kalko 2001; Surlykke and Moss 2000; Brinklov et al. 2010). The same is true for calls emitted at objects that are far away compared to the calls emitted at close objects (Griffin 1953; Matsuta et al. 2013; Jakobsen and Surlykke 2010; Schnitzler 1968; Kalko and Schnitzler 1989; Aytekin et al. 2010). Bats also adjust their call design when orientating in noisy environments (Habersetzer 1981; Ibanez et al. 2004; Miller and Degn 1981). These adaptations may reduce the risk of being jammed. In conclusion, optimal call designs exist for each behavioral situation.

Distance processing

Bats detect, localize, and identify objects with echo information (Wohlgemuth et al. 2016b; Moss and Surlykke 2010; Simmons 1989; Neuweiler 1990). An object's distance represents one important parameter that bats infer from the echoes. The following description mainly focuses on distance processing in FM-bats. Mechanisms of distance processing in CF-FM bats are reviewed in Wenstrup et al. (2012) and Suga (2015).

Two theories have been proposed that explain distance processing by using echolocation information. In 1945, Hamilton Hartridge proposed the "direct time measurement theory" which states that distance is inferred by measuring the echo delay (Hartridge 1945) (Figure 3a). His hypothesis was later confirmed by behavioral experiments (Cahlander et al. 1964). However, determining the echo delay is only possible if call and echo do not overlap with each other. Corresponding to a call duration of one millisecond, distances shorter than 17 cm cannot be determined by using the echo delay. Therefore, a second theory was proposed that allows distance calculations from overlapping call echo pairs. The bat determines distances shorter than 17 cm with the aid of characteristic spectra arising from call echo interferences (Pye 1961a, b; Sanderson and Simmons 2002, 2000; Pye 1960). If a sound wave of the echo is phase shifted by 180° from a sound wave of the call and both sound waves have the same frequency and amplitude, then the two waves cancel out each other (Neuweiler 1990; Simmons 1989). The spectrum arising from the call echo interference has spectral notches or glints at this particular frequency. The positions of the spectral notches along the frequency axis depend on the echo delay and can be used to infer the object's distance. In the following description, I will focus on distance calculations that are based on the "direct time measurement theory".

To determine the echo delay, the brain extracts the time points of call emission and echo arrival. In 1963, Alan D. Grinnell recorded neuronal signals from the auditory midbrain of the FM-bat *Myotis lucifugus* (Grinnell 1963b). He identified neurons responding with facilitation to the combination of two signals, meaning that the response is stronger than the summed response to call and echo alone. By varying the delay between the two signals, the neurons' spike rate varies. Thus, the neurons encoded the delays with their spike rate. The echo delay evoking the strongest response is referred to the best delay (Kössl et al. 2015). Delay-tuned neurons have been described in the auditory cortex (AC) and auditory midbrain of different FM-bat species (Sullivan 1982a; Feng et al. 1978; Dear and Suga 1995; Hagemann et al. 2010; Dear et al. 1993b; Dear et al. 1993a).

In FM- and CF-FM bats, delay-tuning is thought to be based on a coincidence-detection mechanism (Sullivan 1982b; Galazyuk et al. 2005; Feng 2011; Casseday and Covey 1996; Wenstrup et al. 2012; Suga 2015) (Figure 3b). It has been proposed that delay-tuned neurons differently process call and echo to fulfill the requirements for a coincidence-detection mechanism. According to the current view, a

paradoxical latency shift (PLS) is necessary to create delay tuning in FM-bats (Hechavarría and Kössl 2014; Galazyuk et al. 2005; Galazyuk and Feng 2001; Sullivan 1982b, a; Feng 2011; Ma and Suga 2008; Klug et al. 2000; Berkowitz and Suga 1989; Wang et al. 2007a). Delay-tuned neurons show an increase in latency with increasing sound pressure level of the stimulus. Thus, the excitatory response to a call is delayed in comparison to the response to the echo because the call is usually louder than the echo. If the excitatory response to the call is delayed by the same amount as the echo delay, then the delayed excitatory response to the call temporally coincides with the excitatory response to the echo (Figure 3b left). The coincidence results in an excitation reaching the spike threshold, thus eliciting spikes in the delay-tuned neuron. If the echo delay does not correspond to the amount of PLS, then no spikes are elicited in the delay-tuned neuron (Figure 3b right). Some findings indicate that PLS is not necessarily required for creating delay tuning. First, although the quantity of PLSs positively correlates with the neuron's best delay, the values of PLS are not equal to the best delay (Sullivan 1982b; Berkowitz and Suga 1989; Galazyuk et al. 2005; Feng 2011; Hechavarría and Kössl 2014). Second, not all delay-tuned neurons show a PLS (Hechavarría and Kössl 2014). Third, delay tuning is also observed when the animal is stimulated with equally intense call and echo (Figure 3c) (Grinnell 1963b; Hechavarría and Kössl 2014; Beetz et al. 2016b). Thus, a call echo discrimination that is based on intensity differences between call and echo is unlikely. In FM-bats, a call echo discrimination based on spectral differences between call and echo can be excluded because delay tuning can be demonstrated with two spectrally identical FM sweeps (Kössl et al. 2015; Dear et al. 1993b; Hagemann et al. 2010).

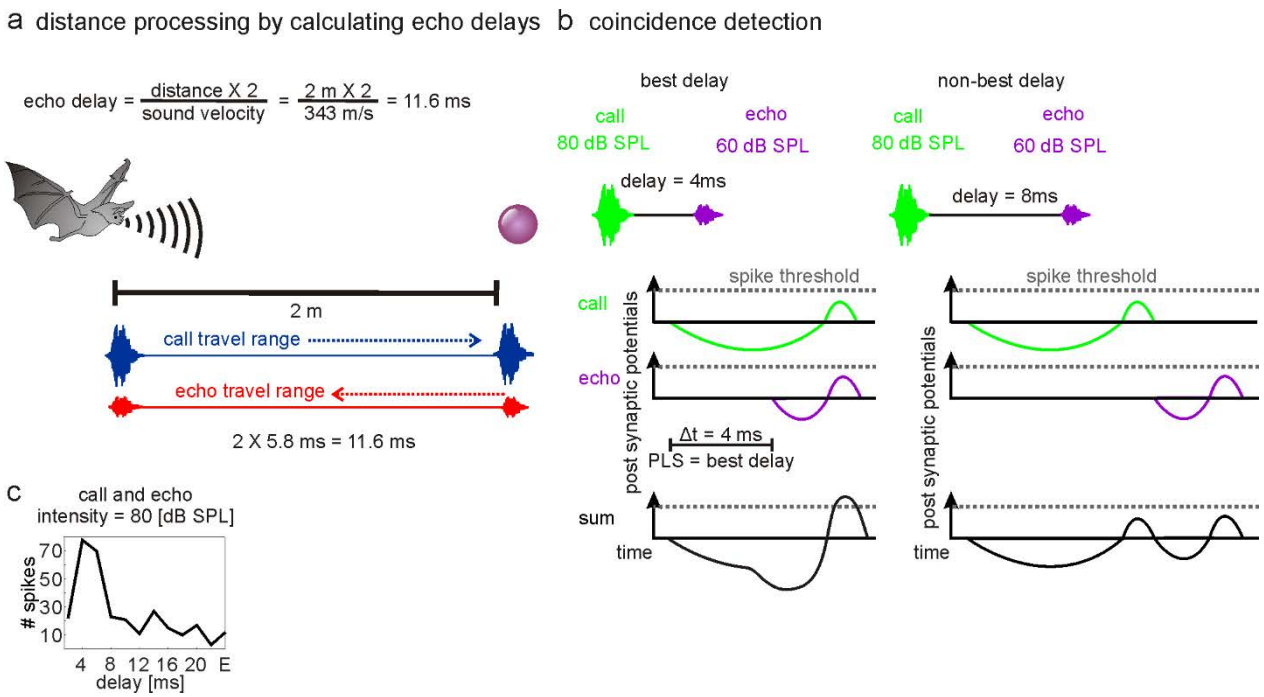

Figure 3 Distance processing based on echo delay information and currently discussed mechanisms of delay tuning in FM-bats. (a) With a call lasting for one millisecond, object distances longer than 17 cm (1 ms echo delay) can be calculated by determining the echo delay. The echo delay represents the time from call onset to echo arrival and depends on the object's distance. According to the sound velocity of 343 m/s, the bat's call hit an object that is 2 m away from the bat 5.8 ms after call emission (blue oscillogram). The echo needs the same duration to travel back to the bat's ears (red oscillogram). Thus, an echo delay of 11.6 ms refers to an object distance of two meters. (b) The current view on the mechanism of delay tuning in FM-bats assumes that delay-tuned neurons do not produce spikes in response to a call (green post-synaptic potential) or echo (violet post-synaptic potential) alone. Call and echo evoke an inhibition that is followed by a subthreshold excitatory rebound. The duration of inhibition is positively correlated with the sound intensity (paradoxical latency shift = PLS). Therefore, the inhibition is longer in response to the call than to the echo. The PLS is approximately as long as the best delay of the neuron (best delay = 4 ms). The delayed subthreshold call rebound coincides with the subthreshold echo rebound. Both rebounds are added to pass the neuronal spike threshold and elicit a spike response. At non-best delays (8 ms on the right side of the subpanel), both subthreshold responses do not overlap and the neuron does not spike. (c) According to the PLS, delay-tuned neurons may not spike when stimulated by equally intense call and echo. However, cortical neurons of *C. perspicillata* show delay tuning when stimulated with equally intense call and echo. For an exemplary cortical neuron, the echo delay is plotted against the amount of evoked spikes in response to pairs of call and echo. *E* stands for echo.

Processing of echolocation signals along the ascending auditory pathway

After reviewing the current knowledge about the mechanisms of distance processing in FM-bats, I would like to describe where and how echolocation signals are processed. Initially, echolocation signals pass through the pinna-tragus system, ear canal, ear drum, and ossicles of the middle ear until they reach the cochlea of the inner ear. At the cochlear level the frequencies of the echolocation signals are represented as a place code (cochleotopic or tonotopic representation) which is common for all mammals investigated so far (von Békésy 1960; Shamma 2001). High and low frequency signals excite primary

auditory nerve fibers that innervate the base and apex of the cochlea, respectively. The frequencies are homogenously represented along the cochlear length (Wittekindt et al. 2005; Kössl 1992). Thus, in comparison to CF-FM bats which possess an auditory fovea at the cochlear level (Suga and Jen 1977), FM-bats do not have an auditory fovea. A downward frequency modulated echolocation call (Figure 4b) sequentially excites auditory nerve fibers tuned to different frequencies. According to the call spectrogram, auditory nerve fibers tuned to high frequencies are excited before the ones tuned to low frequencies. The echolocation call elicits an activity wave travelling from the base towards the apex of the cochlea (Suga 1964). Anatomically, auditory nerve fibers project to the cochlear nucleus. From the cochlear nucleus, the auditory signal diverges into parallel processing auditory pathways of the auditory brainstem (Vater et al. 1997). In these pathways, different auditory centers, such as the lateral lemniscus, superior olive, and the trapezoid body, are involved. In the auditory brainstem, relatively large synapses maintain a high temporal spiking precision and allow a one-to-one spike transmission (Vater et al. 1997). A temporally precise and reliable neuronal response is required to time stamp the onsets of call and echo. From the auditory brainstem, the pathways converge at the level of the auditory midbrain. The auditory midbrain, or inferior colliculus (IC) is tonotopically organized. In the IC, the encoded frequency increases with increasing depth (Sterbing et al. 1994; Schmidt et al. 1991; Jen and Chen 1998; Grinnell 1963a). Echolocation signals, whose main energy concentrates at high frequencies, are mainly processed in ventral parts of the IC. Along the ascending auditory pathway, delay-tuned neurons are created at IC level (Galazyuk et al. 2005; Wenstrup and Portfors 2011; Yan and Suga 1996; Portfors and Wenstrup 1999, 2001). The distance information could diverge from the IC to the cerebellum, the pontine grey, the superior colliculus, and to the auditory thalamus, or medial geniculate nucleus (Casseday and Covey 1996). Thalamocortical fibers transfer the distance information from the thalamus to the cortex. The neuronal bandwidth, represented by the range of echo delays evoking fascilitatory responses, decreases from the IC to the thalamus and to the AC (Yan and Suga 1996; Suga and Horikawa 1986; Olsen and Suga 1991). Recent cortical data show that GABA can modify response strength, bandwidth and best delay of delay-tuned neurons (Hechavarría and Kössl 2014). However, it is still unknown to what extent delay tuning gets modified along the colliculothalamic and thalamocortical tract.

In the mustached bat *Pteronotus parenllii,* which is a CF-FM, the distance information is topographically represented from the thalamic level on (Wenstrup 1999; Wenstrup and Portfors 2011; Pearson et al. 2007; Yan and Suga 1996; Suga et al. 1983; Suga 1990). A topographic organization of echo-delays is called chronotopy. Unfortunately, data examining chronotopy in the thalamus of FM-bats are still lacking. However, chronotopy has been found in the cortex of several FM-bat species (Kössl et al. 2014). In the AC, short delay-tuned neurons or neurons encoding short distances innervate rostral regions. Long delay-tuned neurons or neurons encoding long distances innervate caudal regions.

Carollia perspicillata: A member of the ecologically most diverse bat family, the *Phyllostomidae*

Experiments of the present thesis were performed in *C. perspicillata* (Figure 4a). *C. perspicillata* belongs to the family of the *Phyllostomidae*. With 160 species, *Phyllostomidae* represents the ecologically most diverse bat family and belong to the New World bats (Altringham 2011). In addition to the high diet diversity (Altringham 2011; Fenton et al. 1992), *Phyllostomidae* live in different habitats, including the tropics, subtropics, hot and humid lowland forest, cool montane forests, and semi-arid deserts. One morphological characteristic that many *Phyllostomidae*, except the *Desmodontia*, share is the fleshy nose-leaf (Altringham 2011). Echolocation signals are emitted through the nostrils and the bats can actively adjust the vertical sonar beam direction with their nose-leaf (Hartley and Suthers 1987; Vanderelst et al. 2010; Weinbeer and Kalko 2007). Despite being highly diverse, all *Phyllostomidae* are nocturnal, have well-developed visual and olfactory senses, and echolocate (Bloss 1999, Thies et al. 1998).

C. perspicillata is one out of nine species categorized in the subfamily of the *Carollinae*. It lives in evergreen and deciduous forests from Mexico to southern Brazil (Cloutier and Thomas 1992). It eats mainly fruits including *Piper spp*, *Solanum spp*, and *Cecropia spp*, and occasionally nectar, pollen, and insects (Heithaus et al. 1975; Fleming et al. 1972). *C. perspicillata* emits brief multiharmonic downward FM signals (Figure 4b). The calls are usually composed of three to four harmonics with highest energy in the 2nd or 3rd harmonic (Thies et al. 1998). The second and third harmonic typically sweep downward from 80-45 and 120-60 kHz, respectively (Thies et al. 1998). Call intensity can reach values up to 99 dB SPL (Brinklov et al. 2011). The calls are usually shorter than 2 ms (Thies et al. 1998; Griffin and Novick 1955) and the intercall intervals range between 170 and 20 ms (Thies et al. 1998). During an approach flight, *C. perspicillata* decreases call duration and intercall intervals (Thies et al. 1998). Robust frequency or intensity adjustments during an approach flight have not been described, yet.

In the present thesis, neurophysiological experiments were conducted in the IC and AC of *C. perspicillata*. Corresponding to the tonotopic organization of the IC, recordings were obtained from ventral regions which putatively process echolocation signals. Except for the frequency tuning, no further acoustic tuning has been tested so far in the IC of *C. perspicillata* (Sterbing et al. 1994).

The AC of *C. perspicillata* is parceled into the primary AC, anterior auditory field, dorsoposterior field, secondary AC and the two high frequency areas (Esser and Eiermann 1999) (Figure 4f). Neurons tuned to high frequencies can be found in the dorsal high frequency areas and the high frequency regions of the tonotopically organized primary AC and anterior auditory field. Note that each high frequency tuned neuron of the cortex is additionally tuned to a particular echo delay and encodes distance information. Thus, in *C. perspicillata* the remaining echolocation information including the object's position, size, shape, and texture have to be encoded by delay-tuned neurons as well.

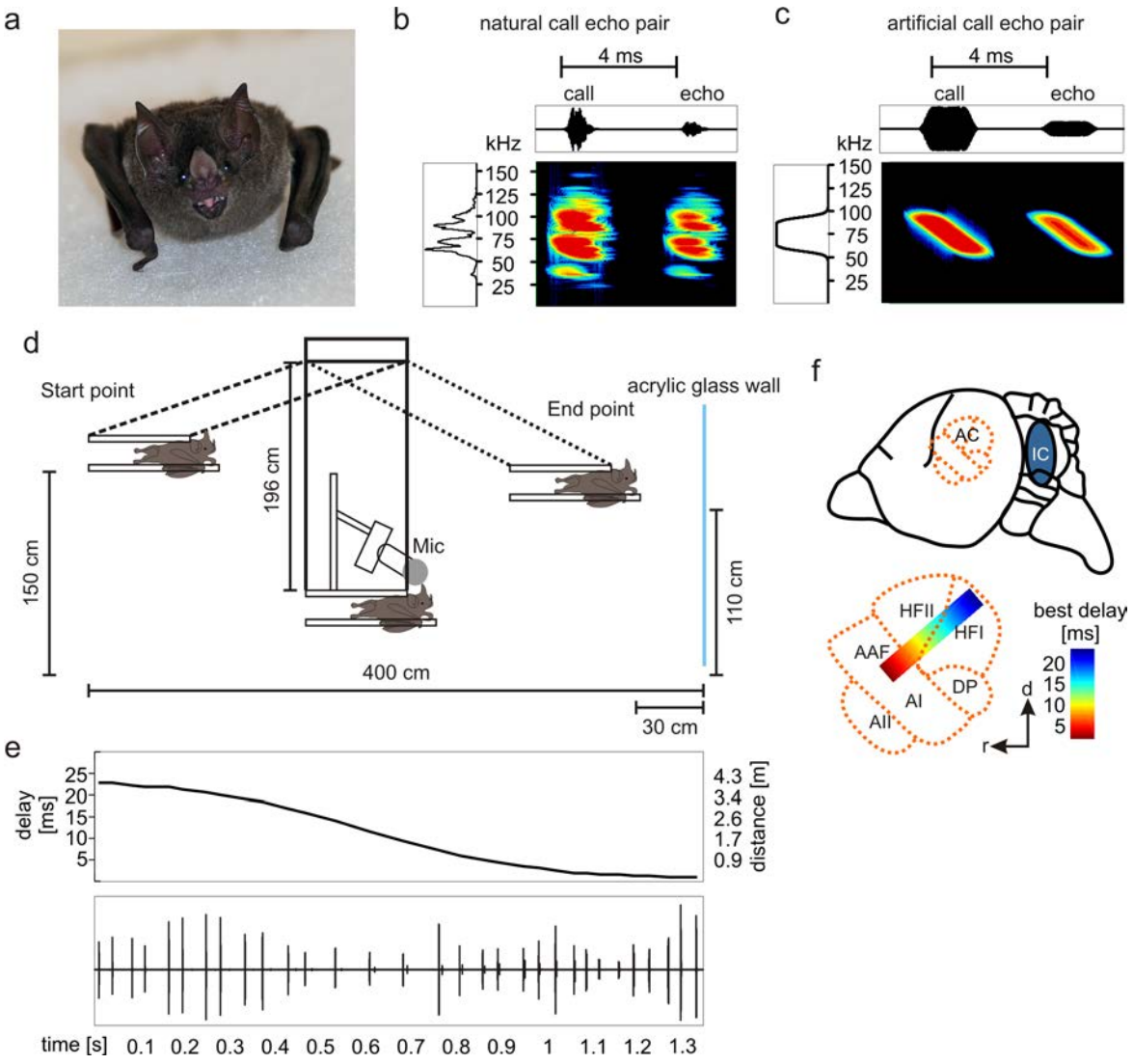

Figure 4 Echolocation behavior and neuroanatomy of *C. perspcillata*. (a) Photo of *C. perspicillata*. (b) Oscillogram, fast fourier transformation (FFT) and spectrogram of a natural call echo pair of *C. perspicillata*. The echo delay is indicated on top. (c) Oscillogram, FFT and spectrogram of an artificial pair of frequency modulated sounds used in previous studies to simulate portions of the natural echolocation signals. (d) Scheme showing the pendulum paradigm from a lateral view. The bat is positioned on a sandwich holder and an ultrasound sensitive microphone (Mic) is attached to the swinging platform. A swing simulates an approach flight to different objects, here an acrylic glass. (e) During the swing the bat emits a sequence of echolocation calls which are reflected off the acrylic glass. Calls and echoes are recorded by the Mic and the echolocation signals are shown as an oscillogram at the bottom. Echo delays decrease according to the object´s distance (top plot). (f) Schematic lateral view on *C. perspicillata*'s brain. The inferior colliculus (IC; blue) and the auditory cortex (AC; orange) which are the brain areas investigated in the present thesis are highlighted. A magnification of the auditory cortex shows the parcellation into its subfields (Esser and Eiermann 1999). Cortical delay-tuned neurons are topographically organized (Hagemann et al. 2010). The best delays decrease from the dorso-caudal to the ventro-rostral axis (color bar). *AI* = primary auditory cortex; *AII* = secondary auditory cortex; *AAF* = anterior auditory field; *d* = dorsal; *DP* = dorsoposterior field; *HFI* = high frequency area I; *HFII* = high frequency area II; *r* = rostral.

Scope of the thesis

To understand how the brain controls behavior, it is necessary to investigate how natural stimuli are processed by the neurons. Neurophysiologists usually use artificial, but well controllable stimuli to investigate neuronal mechanisms of sensory processing. During the electrophysiological recordings, the animals are stimulated by these salient stimuli in the absence of any interfering signals. Although useful, such recording scenarios are far away from any natural condition. The present study tried to stimulate animals under more realistic conditions. Bats are well suited animal models to investigate neuronal processing of behaviorally relevant stimuli. Echolocation signals can easily be recorded from the animals and later can be used as acoustic stimuli. To record the natural stimuli used in the present study, a bat was placed in a pendulum (Henson et al. 1982; Figure 4d) and it was swung towards different objects. During the swing, the bat emits a sequence of calls (Figure 4e). These calls together with the echoes were recorded with an ultrasound sensitive microphone attached to the moving pendulum platform and adjusted as close as possible to the bat's ears. Recorded echolocation sequences and derivatives of them were used as stimuli for electrophysiological experiments. Electrophysiological recordings were conducted either from the IC (Beetz et al. submitted A; Beetz et al. submitted B) or AC (Beetz et al. 2016b, a; Beetz et al. submitted B) in passively listening bats. With this methodological approach different experimental questions were investigated:

1. How does the temporal pattern of the sequence influence the neuronal processing of echolocation signals?

Neuronal activity from delay-tuned neurons have been commonly recorded in response to pairs of artificial, single harmonic FM sweeps (Figure 4c; Kössl et al. 2015; Hechavarria et al. 2013; Hagemann et al. 2010; Dear and Suga 1995; Kossl et al. 2012). These FM pairs were presented at random echo delays and an interstimulus time interval of 400-500 ms. A comparison of the spectrograms between a natural call of *C. perspicillata* and a FM sweep shows that the two signals differ from each other (Figure 4). In *C. perspicillata*, neuronal tuning to natural echolocation signals has thus far never been characterized. To test the influence of the short call intervals on neuronal processing of echolocation sequences, a natural echolocation sequence (Figure 4e) was segmented into its call echo elements. The elements (element situation) and the echolocation sequence (sequence situation) were randomly presented to the bat, with a 400 ms interstimulus time interval. Neuronal signals in response to the element and the sequence situation were recorded from the IC (Beetz et al. submitted A) and AC (Beetz et al. 2016b).

2. How do neurons process echolocation sequences that contain echo information from many different objects?

During echolocation, each object within the surrounding evokes an echo. Therefore, bats navigating in a cluttered environment usually receive multiple echoes in response to each emitted echolocation call (Moss and Surlykke 2010). To test the influence of multiple echoes on neuronal processing, I recorded an echolocation sequence containing echo information from three objects (multi-object sequence) in the pendulum paradigm (Beetz et al. 2016a). Three objects were positioned at different distances along the swing trajectory. In the recorded multi-object sequence, each call was followed by at least two echoes carrying information from different objects. By deleting object specific echoes, I changed the multi-object sequence into sequences that contain echo information from one or two objects (single- and dual-object sequence). By using the multi-, dual-, and single-object sequences as stimuli, I could test the influence of the presence of each object on the neuronal response. Neuronal processing of these stimuli was tested in the IC (Beetz et al. submitted A) and AC (Beetz et al. 2016a).

3. How do the neuronal responses to single- and multi-object sequences change the view on the mechanisms of delay tuning?

Delay tuning mechanisms have never been discussed with respect to neuronal processing of echolocation sequences. By comparing the neuronal responses to the element situation with the ones to the sequence situation, I tested the influence of the temporal pattern of the sequence on the delay tuning. Neuronal responses to the multi-object sequence allow inferring the influence of PLS on call echo discrimination. In the multi-object sequence the first echo that follows the call was always louder than the subsequent echo. If call echo discrimination is based on PLS, then the first echo could erroneously be interpreted as a call and the second echo as a corresponding echo.

4. How do acoustically interfering sounds influence echolocation behavior and neuronal processing of echolocation sequences

Bats often echolocate close to each other which creates an acoustic environment that is full of biosonar signals. For proper echolocation, the bats need to segregate their own biosonar signals from the acoustic background noise. Many behavioral adaptations have been shown that reduce the risk of acoustic interference. However, the value of these adaptations on facilitating echolocation in noisy environments has never been discussed with respect to neurophysiological results. So far, it is unknown how *C. perspicillata* behaves in the presence of acoustic noise. Therefore, I did a behavioral experiment in a flight chamber where the bat had to echolocate in the presence and absence of acoustic signals (Beetz et al.

submitted B). The acoustic signals could interfere with the bat's echolocation system because they represented echolocation signals of the tested bat. This set-up allows characterizing the call emission pattern under silent and noisy conditions. Under noisy conditions, the bat grouped its calls more tightly over time and the minimum call intervals decreased. With neuronal recordings from the IC and AC (Beetz et al. submitted B), I tested whether echolocation information from such a high stimulus rate could still be processed. Alternatively, the enhanced stimulus rate could potentiate suppression effects (Beetz et al. submitted A; Beetz et al. 2016b).

5. How is the topographic representation of echo delays along the cortex affected by stimulation with natural echolocation sequences?

Topographic maps are usually blurry (Kössl et al. 2015; Kaas 1997; Schreiner and Winer 2007). The same is true for the chronotopic map of *C. perspicillata* (Hagemann et al. 2010; Hechavarria et al. 2013). Different aspects can account for blurry maps (for details see discussion). For example, topographic maps are often computed from different animals or at least from many single electrode penetrations. In the present thesis, I characterized chronotopic maps with multi-electrode recordings (Beetz et al. 2016b, a; Beetz et al. submitted B). This approach allows excluding the impact of inter-individual variability and temporal variability of neuronal tuning on the chronotopic map. The use of different naturalistic stimuli further allows testing the generalization and robustness of the map in response to different stimuli.

In summary, the present thesis aims to give an overview on how behaviorally relevant stimuli are neuronally processed in *C. perspicillata*. Neuronal tuning was assessed by stimulating the animals with natural stimuli containing spatial information from one, two, and three objects. The robustness of the neuronal responses to an echolocation sequence was assessed in the presence of different interfering natural acoustic signals. This paradigm should mimic a stimulus situation where many bats echolocate close to each other. Each experimental part of the thesis aims to make the stimulus situation as realistic as possible. The results of the present thesis should motivate researchers to consider more natural stimulus scenarios in future neurophysiological studies.

Results

Temporal tuning in the bat auditory cortex is sharper when studied with natural echolocation sequences.

M. Jerome Beetz, Julio C. Hechavarría, Manfred Kössl

Scientific Reports (2016), 6:29102

Abstract Precise temporal coding is necessary for proper acoustic analysis. However, at cortical level, forward suppression appears to limit the ability of neurons to extract temporal information from natural sound sequences. Here we studied how temporal processing can be maintained in the bats' cortex in the presence of suppression evoked by natural echolocation streams that are relevant to the bats' behavior. We show that cortical neurons tuned to target-distance actually profit from forward suppression induced by natural echolocation sequences. These neurons can more precisely extract target distance information when they are stimulated with natural echolocation sequences than during stimulation with isolated call-echo pairs. We conclude that forward suppression does for time domain tuning what lateral inhibition does for selectivity forms such as auditory frequency tuning and visual orientation tuning. When talking about cortical processing, suppression should be seen as a mechanistic tool rather than a limiting element.

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Was hat der Promovierende bzw. was haben die Koautoren beigetragen?

(1) zu Entwicklung und Planung

Promovierender MJB: 40%
Co-Autor JCH: 40%
Co-Autor MK: 20%

(2) zur Durchführung der einzelnen Untersuchungen und Experimente

Promovierender MJB: 90% Neurophysiologische Ableitungen; Bau von Glaselektroden-Arrays
Co-Autor JCH:10% Unterstützung bei den Ersten Experimenten und beim Bau der Glaselektroden-Arrays

(3) zur Erstellung der Datensammlung und Abbildungen

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Temporal tuning in the bat auditory cortex is sharper when studied with natural echolocation sequences

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M. Jerome Beetz, Julio C. Hechavarría & Manfred Kössl

Precise temporal coding is necessary for proper acoustic analysis. However, at cortical level, forward suppression appears to limit the ability of neurons to extract temporal information from natural sound sequences. Here we studied how temporal processing can be maintained in the bats' cortex in the presence of suppression evoked by natural echolocation streams that are relevant to the bats' behavior. We show that cortical neurons tuned to target-distance actually profit from forward suppression induced by natural echolocation sequences. These neurons can more precisely extract target distance information when they are stimulated with natural echolocation sequences than during stimulation with isolated call-echo pairs. We conclude that forward suppression does for time domain tuning what lateral inhibition does for selectivity forms such as auditory frequency tuning and visual orientation tuning. When talking about cortical processing, suppression should be seen as a mechanistic tool rather than a limiting element.

Precise temporal processing is crucial in the auditory system. It is important for distinguishing phonemes and words in human language[1]. Auditory deficits like "word deafness" are associated with decreased temporal precision at the level of the primary auditory cortex[2,3].

Physiologically, cortical neurons show strong forward suppression[4,5] resulting in decreased ability of temporal tracking[6]. The present study tries to investigate how the echolocation system of bats, and more precisely how cortical neurons tuned to specific target-distances cope with fast auditory stimuli that should induce strong suppression effects.

Bats are animal models suited very well for investigating temporal processing in the auditory system. During echolocation, they broadcast sequences of calls at high repetition rates (Fig. 1a). For example, in the bat species *Carollia perspicillata*, the minimal intercall time intervals are around 20 ms[7]. The bats calculate the distance of objects with the help of the delay of returning echoes and neurons that are tuned to echo-delay are a characteristic feature of auditory cortex of bats[8]. Inactivation of such neurons leads to deficits in echo-delay perception[9]. Although target-distance neurons were discovered in the 1970's, studies on delay tuning so far have focussed mainly on describing neuronal receptive fields by using simulated pairs of biosonar call and echo. Little is known about the responses of delay-tuned neurons when the bat faces natural echolocation streams.

The main goal of the present paper was to describe how the temporal arrangement of information in the biosonar sequences affects the response of cortical delay-tuned neurons. To achieve this goal, we compared the response to natural echolocation sequences with an "expected response", estimated by presenting the single call-echo elements that composed the natural echolocation sequences in a random order and at long (400 ms) inter-call time-intervals. Our results show that strong suppression dominates the response of delay-tuned neurons to natural echolocation sequences. However, the suppression is non-uniform, because responses to specific call-echo elements, whose echo delays represent the neurons' "best" echo delay, are less suppressed than responses to neighbouring call-echo elements. In addition, delay-specific facilitation adds to a sharpening of the neuronal response to the natural echolocation sequence.

Results

Natural echolocation sequence. A representative echolocation sequence for an approach flight was recorded from a bat that was swung in a pendulum (Fig. 1a, method after[10]) towards an acrylic glass wall. During the swing the animal echolocated and the broadcasted calls and their echoes, arising from call reflections from the wall, were recorded with an ultrasonic microphone located on top of the animal at 4 cm distance to the ears.

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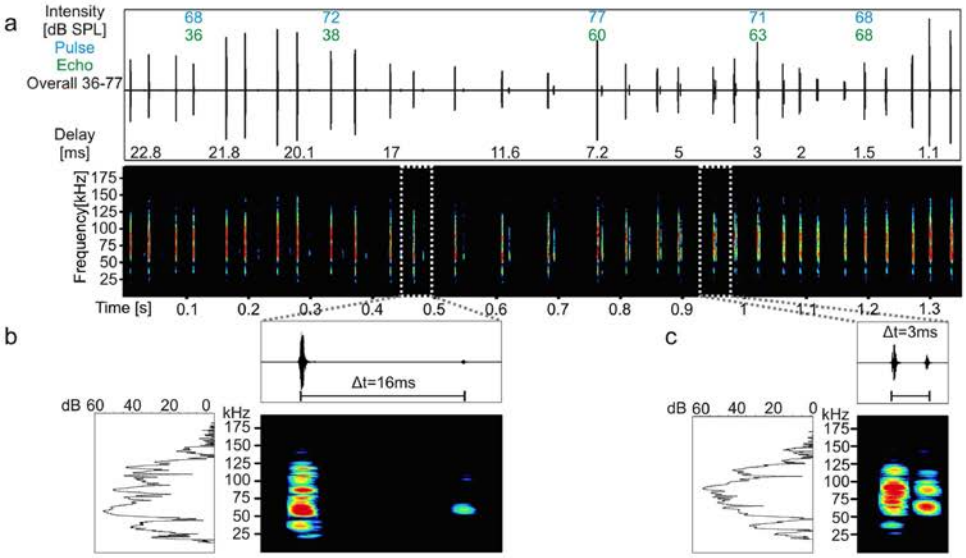

Figure 1. Natural echolocation sequence used as stimulus for electrophysiology recordings. (a) Oscillogram and spectrogram of the echolocation sequence. The sound pressure levels at the maximal level ranged between 36–77 dB SPL. The intensities of selected call-echo elements are indicated in blue and green for call and echo, respectively. Echo delays ranged from 22.8 to 1.1 ms. The temporal delays between call and echo are indicated in the lower part of the oscillogram for selected call-echo elements. **(b,c)** Magnified oscillogram, spectrogram and power spectrum from two example call-echo elements with echo delays of 16 and 3 ms (1024 samples; Hanning window). See also Supplementary Table 1.

The echolocation sequence used as natural stimulus, consisted of 31 call-echo elements. Echo delays decreased from 22.8 ms to 1.1 ms which corresponds to a decrease of object distance between 388 to 19 cm. Magnified call spectrograms (Fig. 1b,c) show that the main energy of the calls was between 50 and 100 kHz (for details see Supplementary Table 1).

Neuronal response of echo delay-tuned neurons to natural echolocation sequence. Electrophysiological recordings were performed with either commercially available tungsten microwire arrays with 16 channels (Tucker Davis Technologies, USA) or with custom-built 4–6 channels glass electrode arrays. The arrays enabled simultaneous recordings from several locations of *C. perspicillata*'s high frequency area of the auditory cortex, where target-distance neurons are located[11]. In total, 149 cortical units (38 units with tungsten microwire arrays and 111 units with glass electrode arrays) were recorded from 10 anesthetized animals from both brain hemispheres (5 females, 5 males). The frequency tuning curves were characteristic for delay-tuned neurons[11], with high sensitivity at around 60 kHz (Fig. 2a). Additionally, the units responded selectively to specific echo delays when they were stimulated with pairs of downward frequency modulated sounds (mimicking the second harmonic of *C perspicillata*'s call) and respond weakly to single downward frequency modulated sounds (Fig. 2b). Delay tuning curves calculated with natural call-echo elements resulted in comparable delay tuning.

To investigate the relevance of the temporal context of the stimuli, we split the echolocation sequence into 31 call-echo elements (Fig. 2c; red vertical lines in the oscillogram indicate time borders) and played them in a randomized fashion with 400 ms interstimulus time intervals. This paradigm is addressed as the "element situation" in contrast to the "sequence situation" in which the echolocation sequence was played in its natural form. Natural stimuli were played at three different sound levels thus covering sound pressure levels that were classified as: (i) high: 36–77 dB SPL, (ii) medium: 26–67 dB SPL; and (iii) low: 16–57 dB SPL (for details see Supplementary Table 1). The spike-times obtained in response to each element in the "element situation" were temporally aligned to the time point of that element in the echolocation sequence (Fig. 2c, bottom: colored raster plots) to create an expected response.

In the "sequence situation" we observed that the firing rate of the units was lower than that observed in response to the "element situation" (compare black (middle) versus colored (bottom) raster plots in Fig. 2c). Some units showed an initial response to the first sequence elements after about 40 ms before they were strongly suppressed (Fig. 2c; red arrow in raster plot). Such initial responses occurred in 68.5%, 69.1% and 57.1% in the recordings obtained at high, medium and low sound levels, respectively. The initial response was not crucial for inducing suppression. In the example given in Fig. 2c, for the medium sound level of 26–67 dB SPL, strong suppression was evident despite a lack of initial response.

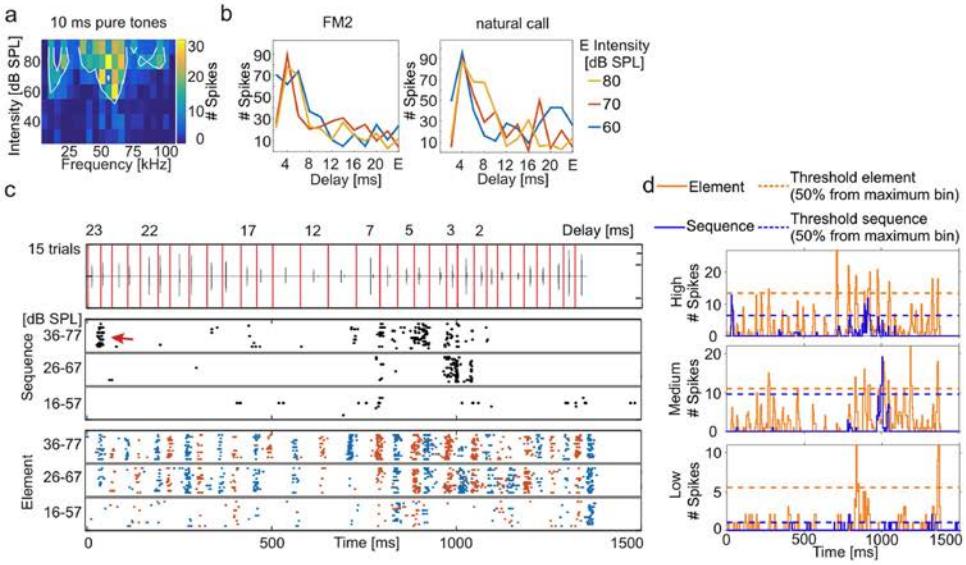

Figure 2. Responses of one example unit to temporally isolated call-echo elements and to the natural echolocation sequence. (a) Frequency tuning curve calculated from responses to different intensity-frequency combinations of a pure tone. The tuning curve demonstrates that the unit is sensitive to high frequencies. **(b)** Delay tuning curves calculated from neuronal responses to artificial frequency modulations that simulated the second harmonic of the bats' calls (left) or to natural call-echo pairs (right). Different combinations of call-echo delays (2–22 ms in steps of 2 ms) and echo sound levels (80, 70 and 60 dB SPL) were presented to the animal. The interstimulus time interval was set to 400 ms. The delay tuning curves show that the unit responded strongly to short delays at around 4 ms. Responses to the echo alone [E] without preceding call is given in the last vertical bins of the data field. **(c)** Top: Oscillogram of the echolocation sequence. Vertical red lines indicate time borders between different call-echo elements that were used for segmenting the sequence. Bottom: Raster plots calculated from the response to the echolocation sequence (black dots) and to the elements (colored dots), where the call-echo elements were randomly presented with a 400 ms interstimulus time interval. To visualize which spikes were elicited in responses to which call-echo element, the responses to consecutive call-echo elements are indicated through alternating colors. Red arrow points to a characteristic initial response. Horizontal gray lines separate raster plots obtained at three sound levels. **(d)** Post-stimulus time histograms (PSTHs; binsize = 5 ms) for the three sound levels and for the sequence (blue) and element (orange) situation. Note that the response to the sequence is suppressed resulting in temporally sharper responses than those in the element situation. Horizontal dashed orange and blue lines indicate thresholds (50% from maximum bin). See also Supplementary Fig. 1.

During the "sequence situation", the overall suppressive effect was strong, however, the units were not suppressed for the entire duration of the sequence. Instead, at some time points, the units recovered from suppression and responded more selectively to specific call-echo elements than in the "element situation". The suppression and sharpening effect can be viewed directly by comparing the post-stimulus time histograms (PSTHs; binsize = 5 ms) from the example unit (Fig. 2d). Note that in this particular unit, a temporally sharp response to certain portions of the echolocation sequence was also evident in response to other natural and artificial echolocation sequences (Supplementary Fig. 1).

Quantification of the response suppression. For quantifying the strength of suppression, we calculated a suppression rate. The suppression rate represents the ratio between the total number of spikes fired in the "sequence situation" (Fig. 3a; black raster plot and Fig. 3b blue PSTHs) and in the "element situation" (Fig. 3a; colored raster plot and Fig. 3b orange PSTHs) and subtracted the obtained ratio from 1. Thus, a suppression rate of 0.91 (Fig. 3a; high sound level) indicates that 91% less spikes occurred in the "sequence" than in the "element situation". Despite of having variable suppression rates, all 149 units studied were suppressed in the "sequence situation" when compared to the "element situation". For example, pooled suppression rates were well above 0, which is the value that would indicate no suppression (Fig 3c.) The suppression rates decreased significantly with sound level (Fig. 3c, Friedman and Dunn's multiple comparison post hoc test; $***p < 10^{-5}$; n = 149).

For quantifying the impact of the suppression on the neuronal tuning, we calculated the 50% bandwidth from the PSTHs. For each unit, sound level and stimulus situation we used a different threshold that was set to the 50% from the bin that contained the maximum number of spikes in the corresponding situation. We did not use the

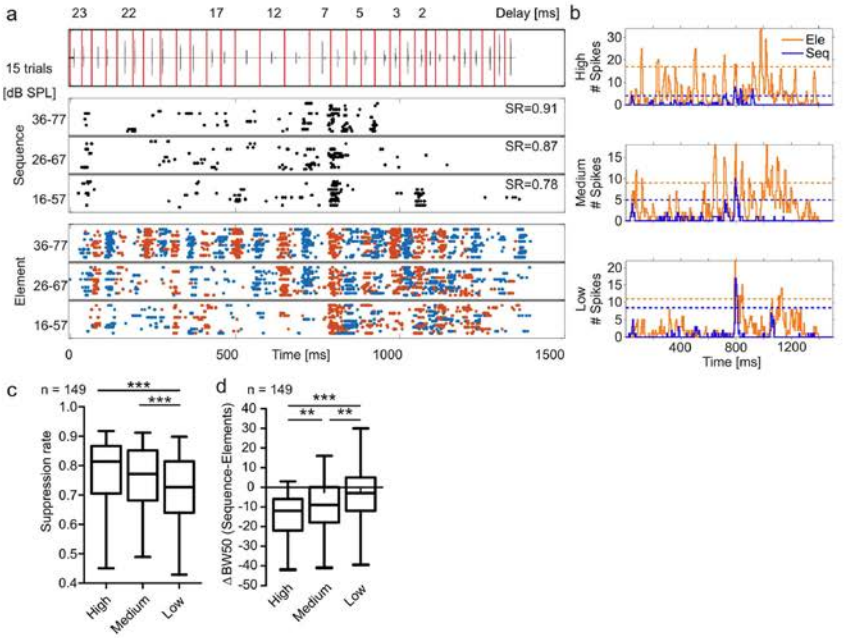

Figure 3. Quantification of suppression induced by behaviorally relevant intercall time intervals. (a) Raster plots of a unit showing sound level dependent suppression. Suppression rate (SR) for each sound level is indicated. **(b)** PSTHs of the unit for each sound level and each stimulus situation (element = orange; sequence = blue). Organization as in Fig. 2. **(c)** Boxplots (whiskers represent 5–95% percentile) showing the suppression rates of all multi-units. **(d)** Boxplots (whiskers represent 5–95% percentile) showing the bandwidth differences between sequence and element situation for each sound level. Friedman and Dunn's multiple comparison post hoc test in **(c,d)** (**$p < 0.01$; ***$p = < 10^{-5}$). See also Supplementary Fig. 2.

same threshold for the "element" and "sequence situation" because in that case, a decreased bandwidth could result from a suppression that is equally strong over time, and which will not represent a sharper delay tuning (Figs 2d and 3b; dashed horizontal lines in the PSTHs). The number of bins surpassing the threshold were compared in the "element" and "sequence situation". As expected, the units were more sharply tuned in the "sequence" than in the "element situation", as indicated by a lower number of bins surpassing the threshold in the "sequence situation" (Fig. 3d). The sharpening effect decreased with decreasing sound level (Friedman and Dunn's multiple comparison post hoc test; **$p < 0.01$; ***$p < 10^{-5}$; $n = 149$). For the highest sound level, a linear correlation between the suppression and the sharpening effect was observed (Spearman $r = -0.32$; ***$p < 10^{-5}$; $n = 149$; Supplementary Fig. 2).

Suppression improves the topographic organization of echo-delays. Cortical delay-tuned neurons are topographically organized in the rostro-caudal direction forming a chronotopic map[11] in which shorter delays are represented rostrally and longer delays more caudally. Simultaneous recordings from six units along the rostro-caudal axis (Fig. 4a) allowed us to observe that the representation of echo delays along the cortical surface is affected by suppression. For example, the chronotopy becomes more distinct when tuning is studied in the "sequence" than in the "element situation" (compare Fig. 4c for "sequence situation" and Fig. 4d for "element situation"). Note that in Fig. 4, the discharge rate from each unit in 5 ms bins was transferred into a color-map, and the units were aligned along the rostro-caudal axis following the electrodes' positions.

For quantifying the neuronal tuning in response to each stimulus situation at the population level we used the time points of all spikes in response to the whole sequence to calculate the median time of the overall response ("median response"; Fig. 4c,d; white dots). We did not use the best response as it has been done in former studies because with the best response only the time point of the maximum response is considered. In contrast the median response is calculated by considering all spikes and their time points of occurrence. When comparing the median responses from each unit for the "sequence" and "element situation", it becomes clear that the median response values cover a larger delay range in the "sequence" than in the "element situation" (Fig. 4e). Thus cortical suppression widens the delay range that the chronotopic cortex is able to respond to. Additionally, in almost two third of the units (96 out of 149), the median response occurred earlier in the "sequence" than in the "element situation" (Wilcoxon signed rank test; $p = < 10^{-5}$, $n = 149$).

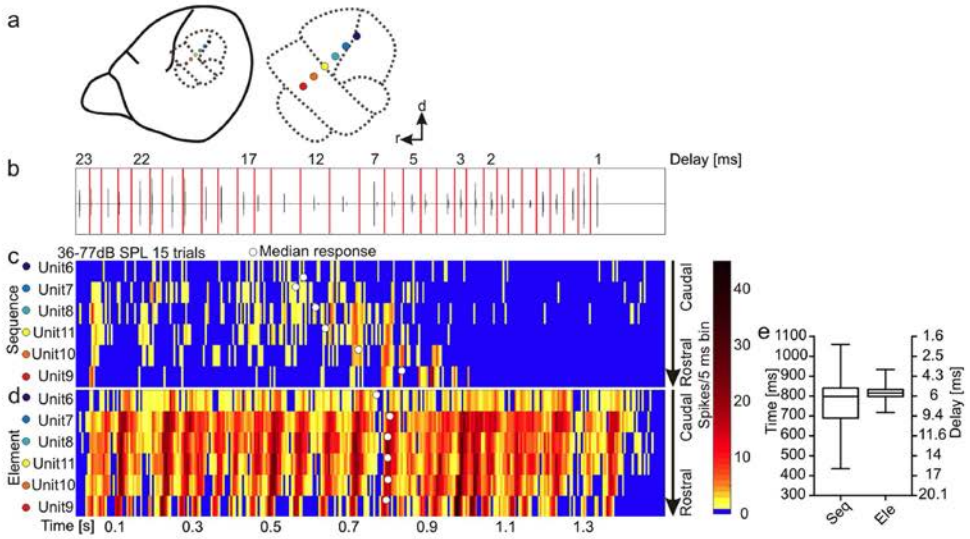

Figure 4. Suppression results in strong topographic organization of echo-delays along the rostro-caudal axis of the brain. (a) Schematic lateral view on *C. perspicillata*'s brain and magnified auditory cortical areas (dashed lines). Colored spots denote electrode positions from a single experiment. The linear electrode array was positioned along the rostro-caudal axis of the high frequency area and high frequency tuned neurons of the primary auditory cortex. d = dorsal, r = rostral. **(b)** Oscillogram of the echolocation sequence. Vertical red lines indicate borders between different call-echo elements that were used for segmenting the sequence. Echo delays ranged from 22.8 to 1.1 ms. **(c,d)** Color-maps with a binsize of 5 ms show the activity pattern of six multi-units in response to the "sequence" and "element situation" at the highest sound level. Each row represents the activity pattern from one unit in response to 15 averages. The units were recorded simultaneously from the auditory cortex and their positions follow the chronotopy along the rostro-caudal axis of the cortex (**a**). Note that the suppression leads to sharper delay tuning and to a more distinct topographic organisation along the rostro-caudal axis in the "sequence situation" **(c)**. In the element situation, the chronotopy is hardly detectable **(d)**. White dots in the color-maps represent time point of the median response for each unit. **(e)** Boxplots (whiskers represent minimum and maximum values) showing the distribution of median responses in 149 units. Note that more delays are covered in the "sequence" than in the "element situation". Thus at the neuronal population more delays are represented in the "sequence situation".

Non-uniform suppression and facilitation shape cortical tuning. For assessing the time course of suppression and facilitation we calculated suppression/facilitation curves by subtracting the PSTHs in the "element" from the PSTHs in the "sequence situation" in a bin-wise manner. Usually, strong suppression was in close temporal vicinity to strong responses (Fig. 5a and supplementary Fig. 3a). At the population level, maximal suppression occurred a few hundred milliseconds after the best response (median = 215 ms, 200 ms, 170 ms for high, medium and low sound levels, respectively; Fig. 5b; Sign test; $^{***}p < 10^{-5}$; n = 149, that indicates a distribution of values whose median deviates from zero).

The best delay of each unit was determined from its response to the "sequence situation" presented at the highest sound level. Best delay was calculated by relating the timing of the maximum response (that is, the bin with the highest spike count) to the call-echo delay occurring right before that time-point (see also Supplementary Fig. 3 for data on medium sound levels). Calculating "best delays" allowed us to relate the neuronal response and strength of suppression to the temporal tuning properties of the neurons.

To visualize the response patterns of all 149 units, normalized neuronal responses (normalized to the maximum spike count observed at the time bin of maximum responses) were ordered in decreasing order according to their best delay so that each row of Fig. 5c–f represents activity from one unit. This figure reveals that the pattern of overall excitation in the "element situation" (Fig. 5c) was matched by a similar pattern of broad suppression (Fig. 5d). Due to the effects of suppression, in the "sequence situation", the temporal tuning was considerably sharper than in the "element situation" (compare Fig. 5e,c). There was an overrepresentation of units responding best to a specific call-echo element representing an echo delay of 7.2 ms (call # 16 in Supplementary Table 1, 22.1%, 33/149 units). Call # 16 had a sound level of 77 dB SPL and its echo, which was delayed by 7.2 ms from the start of the call, had a sound level of 60 dB SPL. Different parameters like sound level, frequency, delay, and stimulus history could be responsible for the observed response overrepresentation to this particular call-echo element.

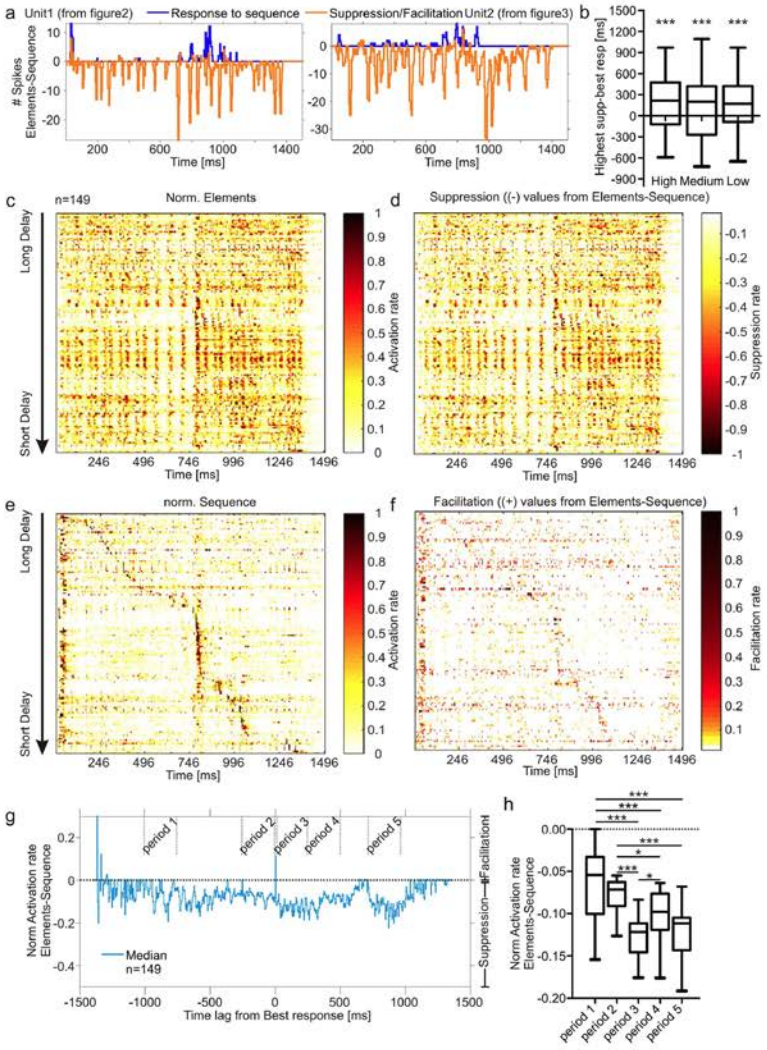

Figure 5. Interaction between non-uniform suppression and facilitation shapes the response to the natural echolocation sequence. (**a**) Response PSTH (blue) from the sequence situation and suppression/facilitation curve (orange) calculated by subtracting the element PSTH from the sequence PSTH, of two units stimulated at the highest sound level. Strong suppression occurs close to strong responses. (**b**) Temporal relationship between best response and strongest suppression for all studied sound levels. Strongest suppression occurs predominantly a few hundred ms after the best response. (**c**) Color-map of normalized response from each unit (organized with decreasing best delays along the y-axis) in response to the "element situation". (**d**) Color-map of normalized amount of suppression from each unit. (**e**) Color-map of normalized response from each unit in response to the "sequence situation". Note that areas of strongest response are much better defined than in (**c**). (**f**) Color-map of normalized amount of facilitation from each unit. Note the facilitation pattern resembles the activation pattern in the "sequence situation". (**g**) Median activation rate curve calculated from temporally aligned contrast PSTHs of each unit. Alignment was based on each neuron's "best temporal bin" and thus best responses correspond to time point 0. The time periods used for statistical comparison are indicated. Each period covers 250 ms. (**h**) Statistical comparison of the median activation rates of the corresponding areas from (**g**). Activation rates are more negative (higher suppression) in the three areas following the best response (period 4, 5 and 6) than in the areas preceding the best response (period 1, 2, 3). Note that (**c–h**) are exclusively presenting data obtained at the highest sound level. Sign test in (**b**). Kruskal-Wallis and Dunn's multiple comparison post hoc test in (**h**) (***$p < 10^{-5}$). See also Supplementary Fig. 3.

In addition to strong, but relatively unspecific suppression, weak facilitation was found right at the best delay of the units (Fig. 5f). The corresponding population pattern matches that of the responses in the "sequence situation". This result emphasizes that the response in the "sequence situation" is shaped through an interaction between widely spread suppression that is strongest after the best response, and facilitation occurring predominantly in the period of the best response.

To better quantify the timing of suppression/facilitation, we pooled the data from the 149 studied units by temporally aligning the normalized suppression/facilitation curves relative to each unit's best response in the "sequence situation" (at the highest level, Supplementary Fig. 3 for data on medium intensity levels). The pooled suppression/facilitation curve shows a clear peak at 0 ms (Fig. 5g). This peak represents the facilitation occurring exactly at the time of the units' best response to the sequence. Strong suppressive periods, indicated by negative values, were detected following the best response until 570 ms and between 715–1000 ms (Fig. 5g). For a statistical analysis, activation rates (calculated as the difference between the response to the sequence and that to the element situations) from three periods (period 3, 4, 5) following the best response were compared with values from two periods preceding the best response (period 1, 2). Each analysed period spanned a time of 250 ms. The activation rates of period 3, 4 and 5 were significantly smaller than the activation rates of period 1 and 2 (Fig. 5h; Kruskal-Wallis and Dunn's multiple comparison post hoc test; $***p < 10^{-5}$; $n = 149$). In other words, this data indicates that the suppression happening after the best response is stronger than that happening before the best response. Note that for medium sound levels, comparable calculations revealed a large suppressive period that also was found after the best response, at between 1–600 ms but at this sound level the facilitation was sparser and temporally highly distributed which resulted in no clear peak at time point 0 (Supplementary Fig. 3).

Impact of stimulus history on neuronal response. The fact that strong suppression usually follows the best response could be an indicator for forward suppression whose strength increases during the stimulation. To clarify the impact of preceding call-echo elements (stimulus history) on the response to consecutive elements, we temporally reversed the echolocation sequence but kept the individual call-echo elements in their original form, so that the call still precedes the echo and both are downward frequency modulated. In other words, the reversed echolocation sequence contains call-echo elements with increasing echo delays over time.

Neuronal activity in response to the natural and to the reversed echolocation sequence ("reversed situation") were recorded. From the example unit in Fig. 6a it is clear that the unit shifted its response towards shorter delays in the "reversed situation" (compare the time ranges of strong excitations for each situation with the help of the colored stars marking specific calls in Fig. 6a). Additionally, more spikes were elicited in the first 500 ms of the "reversed situation" (lower panel in Fig. 6a) than in the last 500 ms of the "natural situation" (upper panel in Fig. 6a). Note that these two temporal windows covered the same call-echo elements in the sequences used as stimuli.

To visualize response pattern differences between the "reversed" and "natural situation" we calculated contrast response curves by subtracting the normalized activity in the "natural" from the "reversed situation". For this subtraction, the PSTH in the "reversed situation" was temporally mirrored which enabled us to temporally align them to the PSTHs obtained from the "natural situation". To exemplify this procedure, one contrast response curve, obtained at the highest sound level, is plotted in Fig. 6b. Positive and negative values indicate more spikes in the "reversed" and in the "natural situation", respectively. For the population data obtained at high sound levels, contrast response curves were calculated for each unit ($n = 129$) and plotted into a color-map (Fig. 6c; see also Supplementary Fig. 4 where exclusively negative (a) or positive (b) values were plotted). In this figure, like in Fig. 5, the units are organized according to decreasing best delays in response to the "natural situation". A median contrast response curve calculated from temporally aligned contrast curves of each unit, comparable to Fig. 5g, is plotted in Fig. 6d. Only bin values differing in the "natural" and "reversed situation" were considered. We observed that at long delays, representing the beginning of the "natural situation" (Fig. 6c,d; beginning from black stars), more spikes were elicited in the "natural" than in the "reversed situation" (Fig. 6e; Sign test for period 1; $***p < 10^{-5}$; $n = 129$). Consistent with the results presented in the previous section, this result suggests the presence of a relatively weak suppression at long delays in the "natural situation" that might arise from a missing stimulus history. In the "reversed situation", a longer stimulus history and therefore a stronger suppression was observed at long delays.

Some units temporally shifted their best response when comparing the response to the "natural" and "reversed situation" (Fig. 6b) but considering the pooled data, the timing of best responses did not shift significantly between the two situations studied (Sign test $p = 0.07$; $n = 129$; median: 805 ms and 765 ms for "natural" and "reversed situation", respectively; Supplementary Fig. 4c). Following the best response in the "natural situation", the neuronal activity was significantly stronger in the "reversed situation" (Fig. 6e; Sign test for period 2; $***p < 10^{-5}$; $n = 129$). The latter could be the result of a stronger suppression operating in the "natural situation" and a relatively weaker suppression occurring in the "reversed situation" (Fig. 6c,d). Based on the different response patterns to the "reversed" and "natural situation", we conclude that the stimulus history, indicated by the different echo delay distribution over time, has strong influence on how the neurons respond to the echolocation sequence. In addition, the fact that suppression increases over the time course of stimulation in the "natural" and "reversed situation", suggests that echolocation sequence processing is strongly influenced by a forward suppression.

Discussion

Temporal processing in the primary auditory cortex is crucial for proper auditory perception including speech[12,13]. Language consists of highly repetitive elements with short time intervals in between. Repetitive auditory stimuli usually lead to cortical suppression and the temporal precision of auditory responses degrades from the periphery towards the cortex[6,14]. How temporal processing quality can be maintained despite cortical

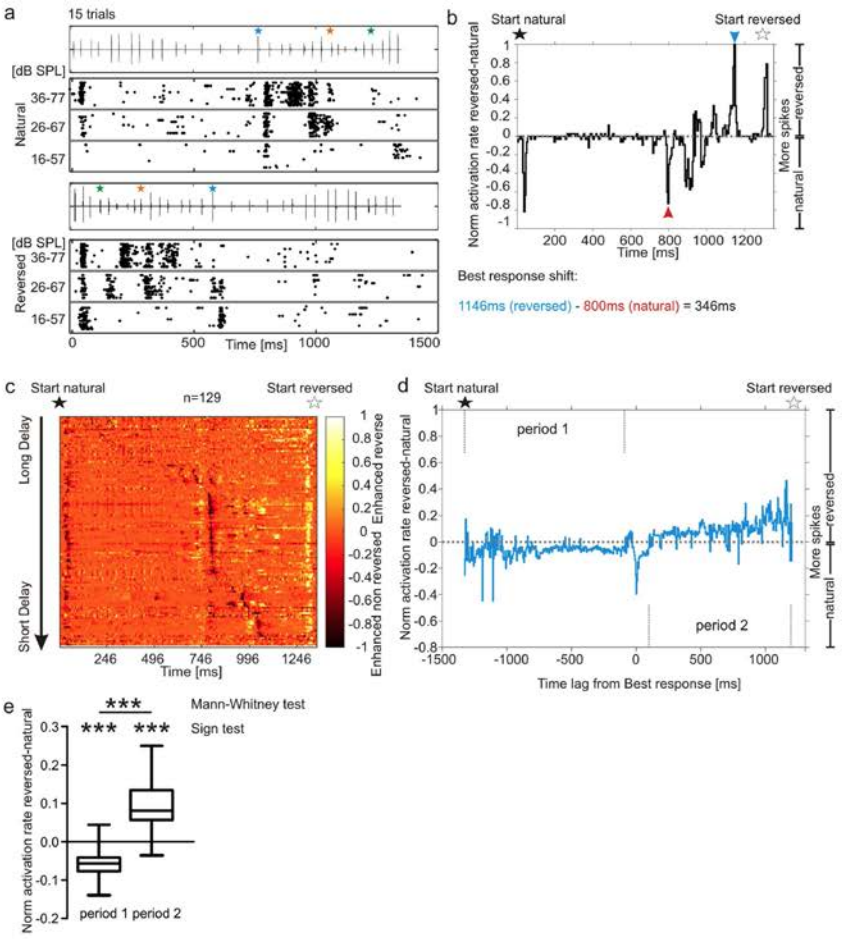

Figure 6. Stimulation with a temporally reversed echolocation sequence indicates a role of forward suppression shaping the time course of cortical responses. (**a**) Stimulus oscillogram and raster plots of a unit stimulated in the "natural situation" (top) and "reversed situation" (bottom). Colored stars signal to specific call-echo elements leading to weaker responses in the "natural" than in the "reversed situation". (**b**) Contrast response curve of the same unit. The normalized PSTH in the "natural situation" was subtracted from the temporally-mirrored normalized PSTH of the "reversed situation". Positive values indicate more spikes while negative ones less spikes in the "reversed" than in the "natural situation". Stars indicate the starting point of the corresponding stimulus (black and white for "natural" and "reversed situation", respectively). Blue and red arrowheads indicate the time point of best response in the "reversed" and "natural situation", respectively. Note that the best response temporally shifts by 346 ms between the two situations. (**c**) Color-map of contrast response curves from each unit (organized with decreasing best delays along the y-axis). (**d**) Median contrast response curve calculated from temporally aligned contrast response curves of each unit. Bins without changes (values = 0) were excluded. Response curves were aligned so that the best responses in the "natural situation" correspond to time point 0. Note that negative values are dominant before and positive values after the zero point. (**e**) Values from period 1 and period 2 in (**d**) have medians that are statistically different from 0 (Sign test; $***p < 10^{-5}$, n = 129) and are also different from each other (Mann-Whitney test; $***p < 10^{-5}$; n = 129). See also Supplementary Fig. 4.

suppression remains elusive. In the present study we wanted to clarify whether cortical suppression is also present in echolocating bats that are specialized for precise temporal coding. Our results show that cortical units of bats are strongly suppressed when they are stimulated with natural echolocation sequences. The sequences had a

behaviorally relevant temporal context and order of call-echo elements (Figs 2–4). Instead of losing the sensitivity to specific call-echo elements due to the suppression, the units were more sharply tuned. The sharper tuning was based on a combination of weak temporally focussed facilitation, and widely spread suppression that is stronger at the time periods that follow the facilitation (Figs 5 and 6).

In the "element situation" the tuning seems to be unusually broad (Figs 2 and 3) when comparing to previous studies that used artificial call-echo elements[11,15,16]. Level relations between call and echo could influence the response strength. Delay tuning curves are often tilted such that with increasing echo level the tuning shifts towards shorter delays[11,15,17,18]. In the natural sequences, each call-echo element has a different level combination thus making it difficult to compare responses in this situation to those observed in more classic delay tuning curves (Fig. 2 and refs 8,19 for review). A second reason for having a broader tuning in the "element situation" could arise from spectral differences in the call-echo composition. The calls of C. *perspicillata* are spectrally similar to each other (Fig. 1b,c). However, based on the relatively broad frequency tuning of delay-tuned neurons it is conceivable that even small spectral differences in the call-echo composition can affect neuronal tuning (Fig. 1a)[11].

Only a few studies exist trying to explain how temporal precision can be maintained under suppression[20,21]. There are two coding strategies that have been described concerning processing of highly repetitive stimuli. Either the neuronal response directly reflects the temporal structure of the sound stimuli (temporal code) or the neurons show sustained responses as soon as a specific stimulus repetition frequency is reached (rate code)[21–23]. According to our results from the "element situation", the delay-tuned neurons might have the potential of temporally tracking the call-echo elements that compose a given echolocation sequence. However, in response to the "sequence situation", the neurons do not temporally follow the echolocation calls but rather respond more selectively to certain portions of the sequences containing specific echo delays. Based on our results it remains unclear, how cortical neurons overcome the suppression in the "sequence situation" and respond in a highly selective manner to specific call-echo pairs?

Suppression was evident from the beginning of the stimulation stream, regardless of whether the first call-echo pairs did evoke spike activity or not. This is consistent with extracellular recordings from delay-tuned neurons of the cortex of *P. parnellii*[24]. Besides, *in vivo* patch clamp recordings from the auditory cortex of rats have shown that even subthreshold responses are followed by long lasting inhibition that causes a decrease in membrane potential[25]. Our results show that suppression builds up over the time course of stimulation (Fig. 6) until reaching its maximum usually a few hundreds of milliseconds after the best response which is consisting with the idea of inhibitory effects that accumulate over time (Fig. 5). Inhibitory effects in auditory and visual cortices have been shown to be strongest close to the best stimulus parameters[26–29].

Note that in the present study the animals were anaesthetized with a mixture of xylazine and ketamine. The impact of anaesthesia on the cortical suppression described in the present study remains speculative.

The interaction between cortical inhibition and excitation is crucial for proper tuning in auditory, somatosensory and visual centres[16,29–37]. In principle two phenomena have been described to explain how inhibitory inputs can sharpen receptive fields. (i) Inhibitory inputs temporally follow excitatory ones, thus limiting the time window of excitation. (ii) Inhibitory neurons are more broadly tuned than excitatory ones, resulting in sharpening effects based on lateral inhibition[27,28,38,39]. Inhibition is not only crucial for shaping receptive fields but also for forward suppression. Cortical forward suppression has a short lasting component (50–100 ms) reflecting GABA-ergic intracortical inhibition[4] and a long lasting component (more than 100 ms) induced at presynaptic sites of thalamocortical neurons[5]. Stimulus combination sensitivity as it is evident in responses to specific call-echo elemtns depends strongly on an interaction between excitation and inhibition[40]. Cortical delay-tuned neurons receive strong GABAergic input, although this does not seem to be involved in creating delay tuning per se[35]. Whether cortical GABAergic circuitry or subcortical inhibitory interactions are crucial for the suppression reported in the present study remains unsolved. It is possible, that cortical suppression elicits an "iceberg effect" via decreasing the excitability of neurons as it has already been proposed in shaping frequency tuning curves[27,38] and in other sensory cortices[41]. Only stimuli evoking strong responses can overcome suppression and elicit spikes in suppressed neurons. Therefore, non-uniform or nonlinear suppression together with facilitatory effects could increase tuning sharpness (Fig. 5).

In bats, enhanced sharpness of duration and delay tuning based on increasing repetition rates has already been described in several studies at the level of the cortex and auditory midbrain[24,42–48]. The main difference between our experiment and previous studies investigating the impact of stimulus repetition rate on neuronal specificity is in the nature of the sound sequences used for stimulation (i.e. natural (this study) vs. seminatural streams (previous studies)). One previous study compared neuronal responses elicited with call-echo elements with responses to a "semi-natural echolocation sequence"[47]. This approach rendered results that are comparable to ours, but the echolocation sequence that they used as stimulus was fundamentally different from the one of the present study. For example, their "semi-natural echolocation sequence" was composed of one natural call repeated with a constant call intensity and intercall time interval of 83.3 ms (12 Hz). However, spectrotemporal call parameters, intensities and intercall time intervals are not constant during an approach flight (Fig. 1). Despite differences in the stimulus settings, the study by Bartenstein[47] reported a sharper delay tuning at the cortex of *Phyllostomus discolor*, an evolutionarily close relative of *C. perspicillata* when stimulating with high repetition rates. In comparison to their results, where only 50% of cortical units were more sharply tuned in response to the "semi-natural sequence", all units (n = 149) of the present study were suppressed in the "natural situation". Based on these differences, one could argue that suppression effects could be stronger when studied with natural (as opposed to seminatural) echolocation sequences. Supporting this idea, a recent electrophysiological study showed stronger selectivity to natural than to artificial echolocation streams in subcortical neurons of the big brown bat (*Eptesicus fuscus*)[49]. These results, together with ours show that the temporal context and spectrotemporal profile of the call-echo elements affect the response selectivity. Strong selectivity to natural sequences is already present at the level of

the midbrain (specifically the superior colliculus). It remains elusive whether the sharper tuning reported here is the result of cortical suppression only or whether it profits from suppression effects that are inherited from subcortical structures. Note that suppression built in the cortex could also shape responses measured in subcortical structures, because of corticofugal projections[50,51].

Regardless of its origin, our results show that cortical neurons can profit from forward suppression which is induced in response to natural echolocation sequences. Suppression acts as a physiological filter that operates in the time domain and that ensures sharp target-distance tuning and a more distinct topographic organization of echo delays. In addition, suppression increases the delay processing range that is covered by the neurons (Fig. 4). Therefore, suppression should be seen as a mechanistic tool rather than a limiting element in cortical processing. Bats are well suited animal models for investigating cortical suppression, because these animals usually encounter highly repetitive stimuli of specific behavioural relevance. In this regard, bats may provide the means to resolve the resolution-integration paradox stating that neurons can either integrate information over long time periods or maintain precise temporal resolution[52].

Methods

Animals. Electrophysiological experiments were performed in ten adult bats (5 females and 5 males) of *Carollia perspicillata* bred in a colony of the Institute for Cell Biology and Neuroscience (Frankfurt University). The animal use complies with all current German laws on animal experimentation and it is in accordance with the Declaration of Helsinki. All experimental protocols were approved by the Regierungspräsidium Darmstadt (experimental permit # F104/57).

Recording of echolocation signals and their preparation for neurophysiology stimulation. For recording natural echolocation sequences, the bat was fixated in a pendulum[10]. An ultrasound sensitive microphone (CM16/CMPA, Avisoft Bioacoustics, Germany) was medially positioned above the animal's head. The microphone was adjusted as close as possible to the ears (~4 cm) to measure the calls and echoes as they would reach the ears of the animal. Its membrane was directed towards the forward swing trajectory. The microphone had a sensitivity of 50 mV/Pa and an input-referred self-noise level of 18 dB SPL. It was connected to a sound acquisition system (UltraSoundGate 116Hm mobile recording interface, +Recorder Software, Avisoft Bioacoustics, Germany) for sound digitalization at 375 kHz (16 bit precision). For offline analysis, digitalized signals were stored in a computer. The bat was swung (total distance = 4 m) towards a smooth, well reflecting acrylic glass wall (50 × 150 cm). During the swing, the animal broadcasted sequences of calls. A specific sequence representing a variable range of echo delays between 22.8 and 1.1 ms was chosen (Supplementary Table 1) to serve as acoustic stimulus for electrophysiological recordings.

The echolocation sequence was resampled from 375 kHz to 384 kHz and background noise was filtered via "Noise Reduction" (FFT length 256; precision 16) with the software Avisoft SAS Lab Pro (Avisoft Bioacoustics, Germany). The "noise reduction" function of Avisoft SAS Lab Pro filters the noise below a certain threshold in the frequency domain. The echolocation calls, together with its echoes, were above that threshold and therefore their spectro-temporal structure was not affected. Remaining artifacts from background noise were filtered with an elliptic filter (order 8) in the software BatSound (Pettersson Elektronik AB, Sweden). Different "stimulus situations" were prepared and played to the anaesthetized animal. In the "sequence situation" the natural echolocation sequence was presented. In the "element situation" individual pulse-echo elements of the natural echolocation sequence were presented without sequence context and a 400 ms interstimulus time interval. The natural echolocation sequence was segmented into its call-echo elements using a custom-written Matlab script (R2009b) and all call-echo elements as well as the natural echolocation sequence were saved as *wav* files. For investigating the relevance of the call-echo element order in the sequence we temporally reversed the natural echolocation sequence with the "reverse" function of BatSound ("reversed situation"). To ensure that each call was followed by an echo and for preserving the original spectrotemporal properties of the call-echo elements (i.e. that they were downward frequency modulated) successive call-echo elements were temporally reversed. The reversed natural echolocation sequence ("reversed situation") as well as the natural echolocation sequence ("natural situation") were randomly presented to the anaesthetized animal.

During electrophysiological recordings, acoustic stimuli were played at a sampling rate of 384 kHz with an Exasound E18 sound card (ExaSound Audio Design, Canada). Each *wav* file (containing either single call-echo elements or the entire sequence) was multiplied by a fading function in which energy increased/decreased smoothly over a time window of 0.5 ms. The latter ensured that no sound artifacts (clicks) were produced by the speaker when playing the natural files. Additionally, the output of the speaker was recorded when the sound files were played and the spectrogram and oscillograms were inspected to look for possible artifacts (with the software BatSound and Avisoft SAS Lab Pro). We could not observe any stimulation artifacts. The audio signals were transferred to an audio amplifier (Rotel power amplifier, RB-850). The bat was stimulated with a calibrated speaker (ScanSpeak Revelator R2904/7000, Avisoft Bioacoustics, Germany) located 15 cm from the bat's ear. A speaker response curve used for calibration was measured with a ¼-inch Microphone (Brüel&Kjaer, model 4939, Denmark) which was connected to a custom-made microphone amplifier.

Frequency-level receptive fields, were measured using pure tone stimuli of 2 or 10 ms duration (0.5 ms rise-fall time). Used frequencies ranged from 5–95 kHz and the sound pressure levels were between 30–90 dB SPL. Sound levels were adjusted based on the speaker's calibration curve. To be able to average the neuronal response, each frequency-level combination was presented 5 times in a randomized fashion and with a 400 ms interstimulus time interval.

Delay-tuning curves were measured with pairs of downward frequency modulations (FM) of 2 ms duration (0.5 ms rise-fall time). In the artificial FMs, the frequency fell from 99 kHz to 49.5 kHz and had its peak energy at 66 kHz. The FM mimicking the call was kept constant at 80 dB SPL and the FM mimicking the echo was adjusted

from 60–80 dB SPL in 10 dB intervals. Echo delays between call and echo (from FM start to consecutive FM start) ranged between 2–22 ms. Each echo delay - echo sound pressure level combination was presented between 15–20 times in a randomized fashion and with a 400 ms interstimulus time interval. A delay tuning curve was also calculated using an example natural call that contained all the echolocation harmonics.

To record and to average the neuronal response each "stimulus situation" ("element", "sequence", "natural" and "reversed") was played randomly 15–20 times at intervals of 400 ms. Three different sound levels were used while presenting the sequence or its elements. The loudest sequence contained elements whose levels ranged between 36–77 dB SPL (Supplementary Table 1). This sequence was attenuated by 10 dB or 20 dB to produce fainter stimulation streams.

Data acquisition and analysis. Electrophysiological recordings took place in a sound-proofed and electrically-shielded chamber. Neuronal responses were recorded in the left and right hemispheres of the bats. For anaesthesia, bats were subcutaneously injected with a mixture of ketamine (10 mg/kg Ketavet, Pharmacia GmbH, Germany) and xylazine (38 mg/kg Rompun, Bayer Vital GmbH, Germany). A longitudinal midline incision was made through the skin overlying the skull. Muscle tissue, covering dorsal and temporal parts of the skull, was removed. A craniotomy above the cortical region corresponding to the high frequency area[53] gave access to auditory neurons. For fixation of the bat's head, a custom-made metal rod (1 cm length, 0.1 cm diameter) was glued onto the skull using dental cement (Paladur, Heraeus Kulzer GmbH, Germany). Each animal was used for chronical recording sessions that lasted up to 8 h over a period of several days.

For electrophysiology, two electrode types were used. (i) commercially available tungsten microwire arrays with 16 electrodes organized in 2 × 8 (Tucker Davis Technologies, USA). The arrays had an electrode spacing of 250 µm and a row spacing of 500 µm. Before penetration, the *dura mater* was carefully removed. Because of the array size, positioning the electrodes initially pushed the cortex downwards, therefore the recordings began only after the cortex had recovered its position (i.e. after 20–30 min, when all the electrodes were already inside the cortex). (ii) custom-built glass electrode arrays of up to 6 channels organized in a row. Glass electrodes (resistance 1–10 MΩ when filled with 3 Mol KCl) were pulled from borosilicate capillaries (GB120F-10, Science Products, Germany) with a Flaming/Brown horizontal puller (P97, Sutter, USA) and they were glued together in a fanshape pattern, ensuring an electrode tip spacing of 250 µm. Neuronal data acquisition used a wireless multichannel recording system (Multi Channel Systems MCS GmbH, Germany) at a sampling rate of 20 kHz per channel and 16 bit precision. Initially we recorded with the microwire arrays but we switched later to the custom-built glass electrode arrays because of several reasons. First, the resistance of the glass electrodes was much higher giving us higher recording quality. Second, recording with glass electrodes was less invasive for the animals because we did not have to remove the dura mater, and therefore each animal could be used for several days. Neuronal responses were analysed in 149 multi-units. 38 units were recorded with the microwire array and 111 units with the glass electrode array. Neuronal tuning was comparable in both recording approaches. For detecting spike events we took a multi-unit specific threshold based on the spike amplitude and used this threshold throughout the recordings for that particular multi-unit. Due to the fact that we used the same threshold within one multi-unit and throughout the stimulation protocol we can confirm that we picked up the same multi-unit activity for each stimulus.

To investigate the impact of the interstimulus time interval of the sequence we compared the neural response to the "sequence situation" and to the "element situation". For achieving this, we realigned the spike-times elicited in response to the individual call-echo elements based on the position of the corresponding call-echo element in the echolocation sequence. For example, the response to the first call-echo element was temporally positioned in the front followed by the spikes elicited by the second call-echo element etc… The result of this realigning procedure was an "expected neural response" to the natural sequence that is based on the responses to the single call-echo elements. For the post-stimulus time histograms (PSTHs) used to process the sequence stimulation data the binsize was set 5 ms. Sign tests were calculated in Matlab (2014) and the remaining statistics in GraphPad Prism 5 (GraphPad Software, USA; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$). The time window for median response calculation ranged from 150 ms after beginning of the sequence to the end of the sequence (1550 ms). The first 150 ms were not considered, to avoid frequently occurring strong initial responses which we considered not to be related to delay tuning.

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Author Contributions
M.J.B. performed experiments. J.C.H. wrote scripts for recordings. J.C.H. and M.J.B. wrote scripts for analysis. M.J.B. analysed data. M.J.B. wrote manuscript. M.J.B., J.C.H. and M.K. conceived and directed the study. All authors discussed the results and commented on the manuscript.

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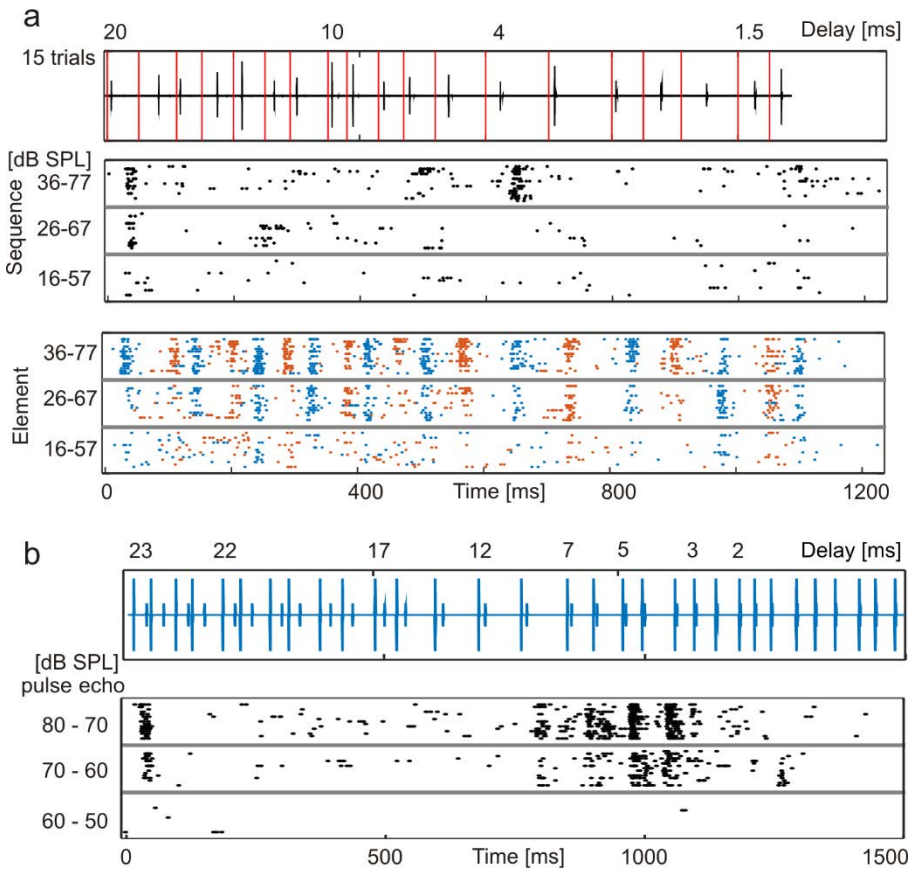
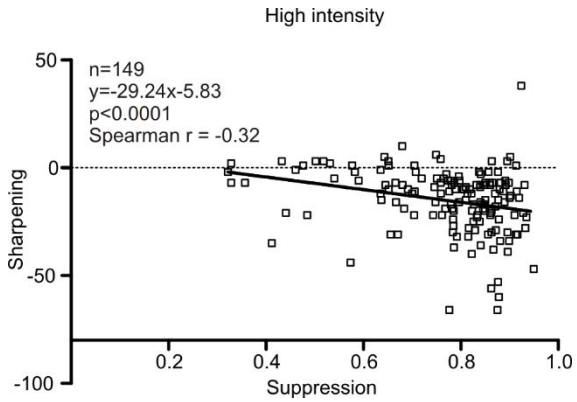
Supplements

Supplementary Table 1 Sequence paramters (refers to Fig. 1)
Calculations were done with Avisoft SASLab Pro (Avisoft Bioacoustics, Germany).
BW = bandwidth; dur = duration; duty cycle = call duration/call interval; f = frequency; int = intensity; rms = root mean square

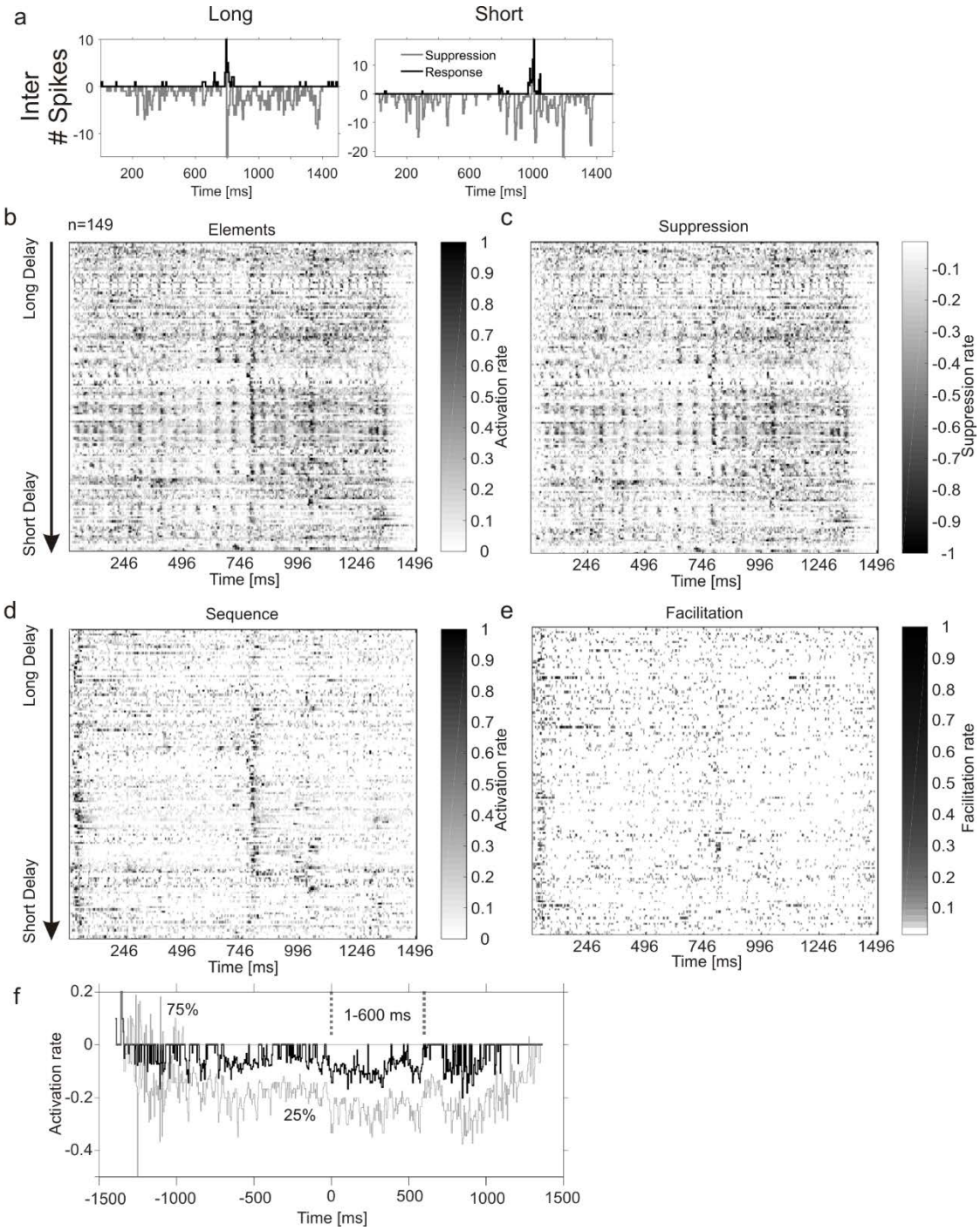
# call	dur	pulse interval	delay	int pulse	int echo	duty cycle	peak f	min f	max f	BW
	[ms]	[ms]	[ms]	rms [dB SPL]	rms [dB SPL]	[%]	[kHz]	[kHz]	[kHz]	[kHz]
1	0.87		22.8	63	38	2.94	76	65.6	89.1	23.4
2	1.073	29.62	22.7	72	36	2.46	77.6	59.6	94.9	35.3
3	0.839	43.573	22.2	69	36	2.94	77.2	62.4	94.5	32
4	0.813	28.573	22	68	36	1.53	71.6	59.6	94.6	35
5	0.766	53.099	21.8	72	36	2.52	76.3	64	92.4	28.3
6	0.896	30.422	21.4	73	40	1.72	73.9	58.9	95.8	36.8
7	0.906	51.964	20.8	73	36	2.83	77.5	65.9	91.7	25.7
8	1.109	31.974	20.1	75	38	2.03	69.4	59.8	91.2	31.3
9	0.901	54.594	19.2	72	38	2.33	77.2	60.1	92.6	32.4
10	0.885	38.688	18.4	77	49	1.55	60.6	55.3	83.5	28.2
11	0.932	57.083	17	74	47	2.50	70.7	56.9	86.9	29.9
12	0.807	37.286	15.9	71	49	1.20	56.7	52.6	80.8	28.2
13	0.698	67.036	14	73	48	0.92	60.1	54.6	80.7	26.1
14	0.849	75.818	11.6	72	57	1.14	57.1	53.8	74.5	20.6
15	0.948	74.385	9.4	73	58	1.19	59.8	54.6	85.5	30.9
16	1.047	79.75	7.2	77	60	2.29	72.5	60	94.4	34.4
17	0.792	45.802	6	69	56	1.55	77.4	63.3	93.8	30.5
18	0.677	50.974	5	65	53	2.01	82.9	62.3	95.8	33.5
19	0.745	33.677	4.3	66	56	1.31	86.9	72.7	94	21.2
20	0.703	57.016	3.4	65	50	2.09	89.9	77.3	94.5	17.1
21	0.687	33.698	3	63	56	1.85	89.8	79.7	94.5	14.8
22	0.823	37.146	2.5	71	63	1.99	90.6	79.1	94.3	15.2
23	0.677	41.266	2	66	66	2.53	90.8	79.9	94.4	14.5
24	0.63	26.708	1.8	65	70	2.21	91.2	76.3	94.2	17.8
25	0.495	28.516	1.6	62	67	1.12	83.5	64.9	98.5	33.6
26	0.516	44.062	1.5	63	63	1.58	82.8	64.6	92.5	27.9
27	0.62	32.667	1.3	68	68	1.79	83.5	76.4	94.3	17.8
28	0.552	34.547	1.2	66	73	1.31	90.8	71.1	94.2	23
29	0.651	41.995	1.1	69	76	2.31	84	65.8	92.9	27.1
30	0.667	28.125	1.1	74	76	1.95	83.2	57.9	100.4	42.5
31	0.651	34.141	1.1	74	77		67.9	57.3	93.6	36.2



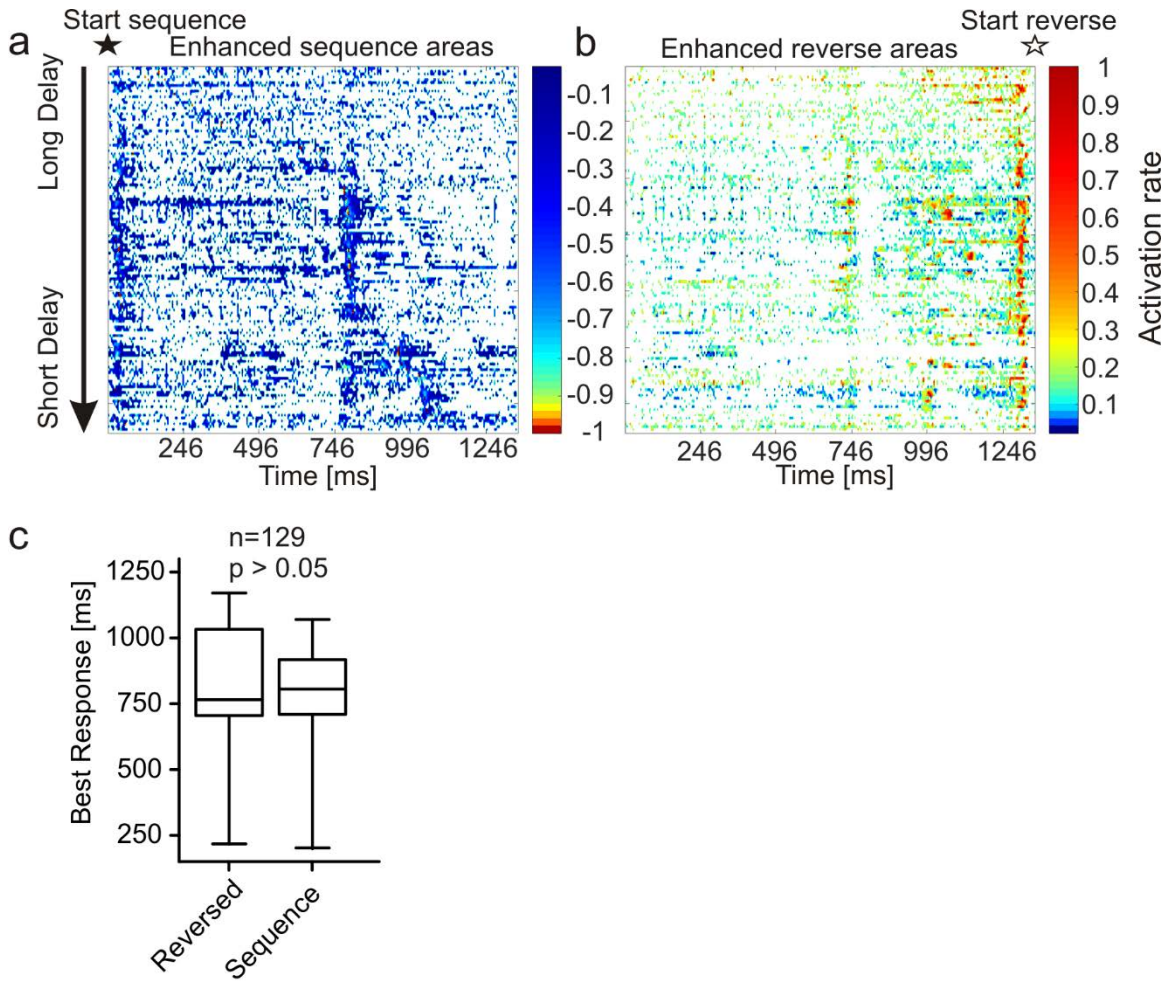
Supplementary Fig.1 Cortical suppression occurring to animal specific echolocation sequence and delay tuning to seminatural echolocation sequence (refers to Fig. 2).
(a) Neuronal response of the multi-unit from figure 2 when stimulated with a "natural sequence" obtained from the animal that was electrophysiologically recorded. Note that the strongest response in the "sequence situation" occurs at 4 ms delay which fits to the tuning properties shown in figure 2b and 2c. Organization of the figure as in figure 2c. **(b)** Neuronal response of the multi-unit to a seminatural echolocation sequence. The sequence was constructed with a natural echolocation call. The call was attenuated by 10 dB and used as an echo. Call and echo positions were adjusted to the positions from the "natural situation" in figure 2. Thus the temporal properties (intercall time intervals and echo delays) were equal to the "natural situation" in figure 2. The intensities of the calls and echoes were consistent throughout the sequence. When comparing the response pattern of **(b)** with that of figure 2c then it becomes clear that the overall response pattern was comparable although the multi-unit responded with more spikes in the "seminatural situation". Intensity differences together with spectral differences of the calls could be responsible for the slightly stronger response in the "seminatural situation".



Supplementary Fig. 2 Linear correlation between the suppression rate and the bandwidth difference at the highest intensity level (refers to Fig. 3). * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$


a
Long
Short
Inter # Spikes
Suppression
Response
Time [ms]
b
n=149
Elements
Long Delay
Short Delay
Activation rate
Time [ms]
c
Suppression
Suppression rate
Time [ms]
d
Sequence
Long Delay
Short Delay
Activation rate
Time [ms]
e
Facilitation
Facilitation rate
Time [ms]
f
75%
1-600 ms
25%
Activation rate
Time [ms]

Supplementary Fig. 3 Forward suppression predominantly occurs right after the best response also at intermediate intensity level (refers to Fig. 5)
(a) Intermediate intensity level PSTH of two example units in response to the natural echolocation sequence (black) and a suppression PSTH (grey). Note that high suppression often occurs close to the strongest response.
(b) Color map of normalized PSTHs from each unit (organized with decreasing best delays along the y-axis) in response to the element situation.
(c) Color map of normalized suppression PSTHs from each unit.
(d) Color map of normalized PSTHs from each unit in response to the sequence situation. Note the extremely sharp activity areas (dark spots).
(e) Color map of normalized facilitation PSTHs from each unit. Note the facilitation pattern resembles the activation pattern in the sequence situation.
(f) Median contrast PSTH calculated from temporally aligned contrast PSTHs of each untit. Alignment occurred, thus best response in the sequence situation correspond to time point 0. Suppressive effects are weaker than in the high intensity level (compare Fig. 5g). One prominent suppressive area at 1-600 ms after the best response occurs. In comparison to figure 5g no peak appeared at time point 0, because of decreased overall activity of the units at that intensity level. Therefore, the median calculation filtered tiny responses out of the median contrast PSTH.



Supplementary Fig. 4 Forward suppression is the prominent suppression form (refers to Fig. 6)
(a, b) Color maps showing exclusively enhanced sequence areas **(a)** and enhanced reverse areas **(b)** bins.
(c) No significant best response shift occurred between sequence and reversed stimulation (Wilcoxon signed rank test: $p = 0.07$)

Cortical neurons of bats respond best to echoes from nearest targets when listening to natural biosonar multi-echo streams.

M. Jerome Beetz, Julio C. Hechavarría, Manfred Kössl

Scientific Reports (2016), 6: 35991

Abstract Bats orientate in darkness by listening to echoes from their biosonar calls, a behaviour known as echolocation. Recent studies showed that cortical neurons respond in a highly selective manner when stimulated with natural echolocation sequences that contain echoes from single targets. However, it remains unknown how cortical neurons process echolocation sequences containing echo information from multiple objects. In the present study, we used echolocation sequences containing echoes from three, two or one object separated in the space depth as stimuli to study neuronal activity in the bat auditory cortex. Neuronal activity was recorded with multi-electrode arrays placed in the dorsal auditory cortex, where neurons tuned to target-distance are found. Our results show that target-distance encoding neurons are mostly selective to echoes coming from the closest object, and that the representation of echo information from distant objects is selectively suppressed. This suppression extends over a large part of the dorsal auditory cortex and may override possible parallel processing of multiple objects. The presented data suggest that global cortical suppression might establish a cortical "default mode" that allows selectively focusing on close obstacle even without active attention from the animals.

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Cortical neurons of bats respond best to echoes from nearest targets when listening to natural biosonar multi-echo streams

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Bats orientate in darkness by listening to echoes from their biosonar calls, a behaviour known as echolocation. Recent studies showed that cortical neurons respond in a highly selective manner when stimulated with natural echolocation sequences that contain echoes from single targets. However, it remains unknown how cortical neurons process echolocation sequences containing echo information from multiple objects. In the present study, we used echolocation sequences containing echoes from three, two or one object separated in the space depth as stimuli to study neuronal activity in the bat auditory cortex. Neuronal activity was recorded with multi-electrode arrays placed in the dorsal auditory cortex, where neurons tuned to target-distance are found. Our results show that target-distance encoding neurons are mostly selective to echoes coming from the closest object, and that the representation of echo information from distant objects is selectively suppressed. This suppression extends over a large part of the dorsal auditory cortex and may override possible parallel processing of multiple objects. The presented data suggest that global cortical suppression might establish a cortical "default mode" that allows selectively focusing on close obstacle even without active attention from the animals.

Animals are continuously exposed to multiple stimuli and their sensory systems should selectively respond to behaviourally-relevant information. Selective attention[1], alternation of fixation points through saccadic eye movements in the visual system[2], detection of rarely occurring stimuli[3–5] and habituation, are some strategies that can account for selective neuronal responses to behaviourally relevant information.

Echolocating bats predominantly rely on their auditory system for short range orientation. Bats broadcast sequences of echolocation calls and they extract relevant orientation cues from echoes that are reflected from surrounding objects[6–9]. Behavioral studies have shown that bats can alternate the direction of their calls. Thus, their focus changes between objects in a saccadic manner resembling saccadic eye movement[6,10]. However, at present, it remains uncertain to which extent bats can spatially restrict their sonar beam. Especially when multiple objects are arranged sequentially at different space depths, a single call could still result in multiple echoes that arrive one after the other to the bats' ears. The mechanisms by which bats can extract object distance information from such natural and complex multiple-object sequences remain obscure.

Bats calculate the object distance with the aid of the echo-delay, which is the time elapsed from emitting a call until the arrival of the corresponding echo. The smaller the echo-delay the shorter the distance to an object. Within the ascending auditory pathway, neurons whose response is facilitated when presented with specific echo-delays can be found as early as in the auditory midbrain[11] and these neurons are defined as target-distance or delay-tuned neurons.

In this study, it is investigated how delay-tuned neurons in the auditory cortex of the fruit-eating bat *Carollia perspicillata* respond to distance information from multiple objects. The main goal was to test whether neuronal responses to multiple-object sequences can be predicted from responses to single-object sequences, created by deleting object-specific echoes in the multiple-object sequences. To achieve this goal we recorded from anesthetized animals while using an echolocation sequence that contained information from multiple echoes as stimulus. The results indicate that only certain portions of the response to multiple-object sequences can

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be predicted accurately from responses to single-object sequences. More specifically, only responses to leading echoes, which originate from the nearest object, are comparable to responses elicited by the same echo in single-object sequences. Responses to temporally lagging echoes that indicate distant targets are suppressed. It is suggested that such selective echo-suppression could be important for "default focussing" of neuronal responses on nearest obstacles. The animals were anaesthetized during the experiments which emphasizes that the selective suppression is a rather basic cortical feature and acts independent of active attentional mechanisms. Processing distance information from nearest objects could be ethologically crucial for evoking fast motor responses for obstacle avoidance during flight.

Results

Neuronal response of echo-delay tuned neurons to multiple-object sequences. For recording a natural multiple-object sequence, a bat was positioned in a pendulum (method after refs 12–14) and it was swung towards three different objects that were separated along the depth axis (Fig. 1a,b). To limit the complexity of the echo spectra three well reflecting objects with smooth surfaces were chosen. Object A had a rock-like shape and it was built out of papier-mâché. Object B was a wooden plate and object C was an acrylic glass wall. Object A was overflown by the animal at about 450 ms after the start of the swing (Fig. 1c, blue vertical line) before the swing stopped directly in front of object B which was positioned 130 cm behind object A. Object C was placed 20 cm behind object B. Object C was larger in height and width than object B, to ensure that object B was not entirely overlapping object C in space and that a faint echo from object C could be detected after the echo of object B.

The broadcasted calls and their echoes, reflected from the three objects, were recorded with an ultrasonic microphone located on top of the animal at 4 cm distance to both ears. The multiple-object sequence consisted of 17 biosonar calls in which each call elicited at least two echoes coming from two different objects (Fig. 1c). Echo-delays that were elicited by call reflections from object A ranged between 11 and 1.7 ms (Supplementary Table 1). Echoes from object B were continuously represented throughout the swing with delays ranging from 18.6 to 1 ms. Echo information from object C covered echo-delays from 14.2 to 8.7 ms. Echoes from object C were elicited as early as the 400 ms after the start of the swing. Note that the late occurrence of echoes from C can be related to the fact that during the first portion of the swing, this object was simply too far away for eliciting echoes that were intense enough for being recorded. Also, object A spatially overlapped with object C, thus, calls that were spatially focused on object A likely could not reach object C. At about 700 ms after the start of the swing, object B spatially overlapped with object C, thus, in this situation no biosonar call was reflected from object C. To explore the impact of each object on cortical processing, single-object-sequences and dual-object sequences were created by deleting object-specific echoes from the multiple-object sequence. The sequences were used as acoustic stimuli in electrophysiological recordings from anaesthetized animals. Note that anaesthesia should prevent attention-dependent effects on the neuronal response.

Regarding the multiple-object sequences, when the bats are stimulated with a call and two echoes coming from two different objects (Fig. 1d), delay-tuned neurons could respond in different ways as illustrated in Fig. 1e. Two different neuron types according to their best delay, could process distance information from both objects (Hypothesis 1 in Fig. 1e). One neuron tuned to 7 ms delay (indicated in red in Fig. 1e) responds to the leading echo and a second neuron tuned to 15 ms delay responds to the lagging echo. Another processing strategy would be that the neuronal population predominantly respond to echoes coming from a single object that produces either leading or lagging echoes (Hypothesis 2). In such a scenario, neurons tuned to a specific echo-delay would respond accordingly to a certain object but the activity of other neurons that could theoretically respond to echo-delays coming from other objects is reduced. In the behaviourally worst case, neurons would respond to the delay between leading and lagging echoes (processing the distances between objects; Hypothesis 3). This processing strategy could lead to misinterpretations since the first echo would be mistaken as a call.

The three possibilities mentioned in the preceding text were tested by analysing neuronal responses from 96 units. Figure 2a–g shows a neuronal response to the sequences consisting of echo information from combinations of objects A, B, and C. The neuronal activity of the same unit in response to other intensity levels is shown in supplementary Figure 1. Delay tuning was assessed based on the time point of the maximum response (best delay) in response to the echolocation sequence that contained echoes from object B only (B sequence; Fig. 2e). The B sequence was used for determining the best delay because that single-object sequence covered the largest delay range from 1 ms–18.6 ms (orange plot in Fig. 1c, *top*). The unit was tuned to short-delays and during the presentation of sequence A and sequence B responded best at 2–3 ms echo-delays (Fig. 2d,e). In the response to the B and C sequence (Fig. 2e,g) the unit did also respond to echo-delays 7 and 13 ms, respectively. These peaks could arise from the fact that delay-tuning strongly depends on the level relationship between pulse and echo, which strongly varied during the sequences[13,15–17]. Usually, the higher the levels are, the wider is the rang of the delay-tuning[14]. Note that in response to the same sequences presented at lower sound pressure levels those peaks in the response were not detected (supplementary Figure 1).

To investigate the impact of echo information from object A on the neuronal tuning, one might can check the neuronal activity in the second half of the sequence beginning from the 450 ms mark. Note that after, the 450 ms mark no echo from object A occurs (indicated by the blue vertical lines in Fig. 2), since at that position the bat had already overflown object A. From that time point on, the multiple-object sequence (Fig. 2a) was equal to the dual-object sequence containing echoes from object B and C (BC sequence; Fig. 2c). The same is true when comparing the AB sequence (Fig. 2b) with the B sequence (Fig. 2e) or the AC sequence (Fig. 2f) with the C sequence (Fig. 2g). Note that both for the ABC versus BC and the AB versus B sequences, the echo composition was the same after the 450 ms mark. Nevertheless, after 450 ms the response to sequences that contained echo information from object A (Fig. 2a,b,f) was weaker than those in the corresponding sequences containing no echo from object A (Fig. 2c,e,g). In other words, it appears that the presence of echoes from object A triggers a suppression that

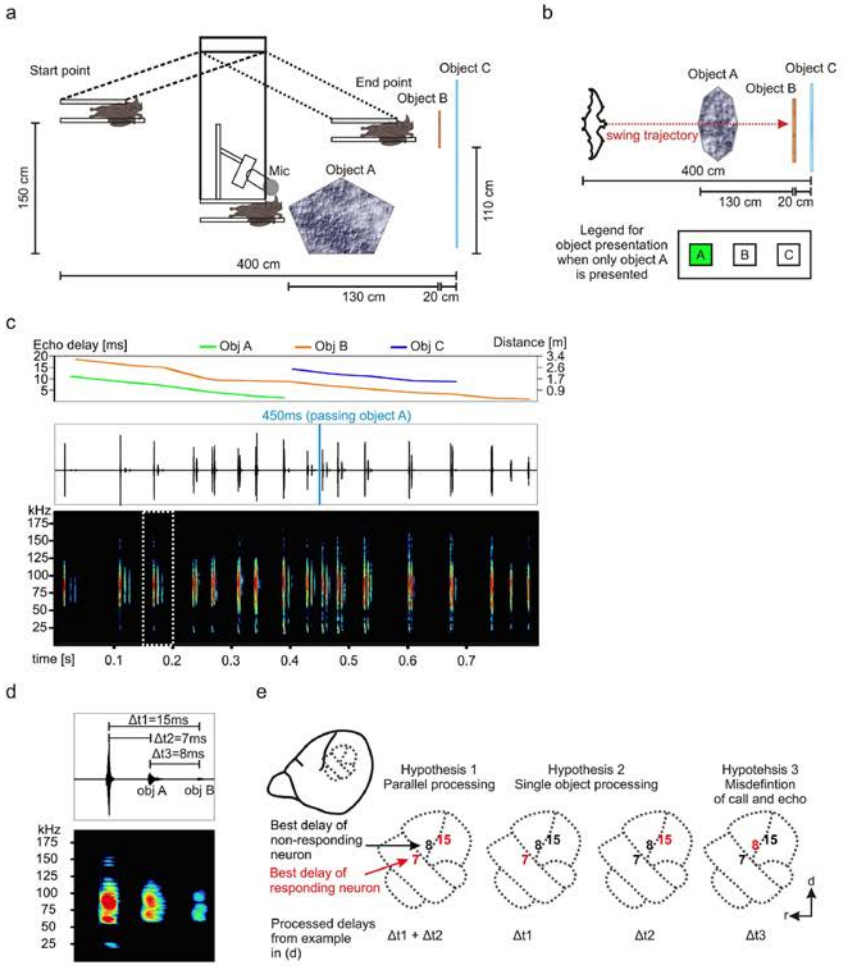

Figure 1. Natural echolocation sequence consisting echo information from three objects. (**a**) Schematic side view (not in scale) of the pendulum paradigm. The animal was fixed in a pendulum and it was swung towards three objects. During the swing the animal echolocates. Biosonar calls and echoes were recorded with an ultrasonic microphone (Mic) that was positioned above the animal's head and it was pointing into the direction of the swing. The distance between the microphone and the animal's ears was about 4 cm. During the swing object A, mimicking the shape of a rock was overflown by the bat. Object B was a wooden plate and object C was an acrylic glass wall. (**b**) Top: Schematic top view (not in scale) of the recording set up. Bottom: Example of a legend indicating a "single-object sequence" in which object A (green filled rectangle) is represented in the echolocation sequence, while echoes from object B and object C (unfilled rectangles) are absent. (**c**) Top: Change of object specific "echo-delays" along the time axis of the sequence. Each object is represented by a differently colored line. Bottom: Oscillogram and spectrogram of the echolocation sequence that was used as acoustic stimulus for electrophysiological recordings. The blue vertical line at 450 ms indicates the time point of passing object A. (**d**) Magnification of one call-echo element marked by white dashed rectangle in (**c**). The echo-delays are indicated in the oscillogram. (**e**) Three hypotheses (1–3) regarding how the echo-delays of the call-echo element in (**d**) could be processed in the cortex of *C. perspicillata*. Schematic lateral view on *C. perspicillata*'s brain and magnified auditory cortical areas (dashed lines). Numbers in the cortical areas represent the best delay to which the neurons respond to. Red numbers indicate responding and black numbers non-responding neurons when the animal is stimulated with the call-echo element based on the corresponding hypothesis. Note that according to hypothesis 3 the delay between two consecutive echoes (Δt3 in **d**) is processed and not the echo-delay between call and echo (Δt1 or Δt2 in **d**). *d* = dorsal; *r* = rostral.

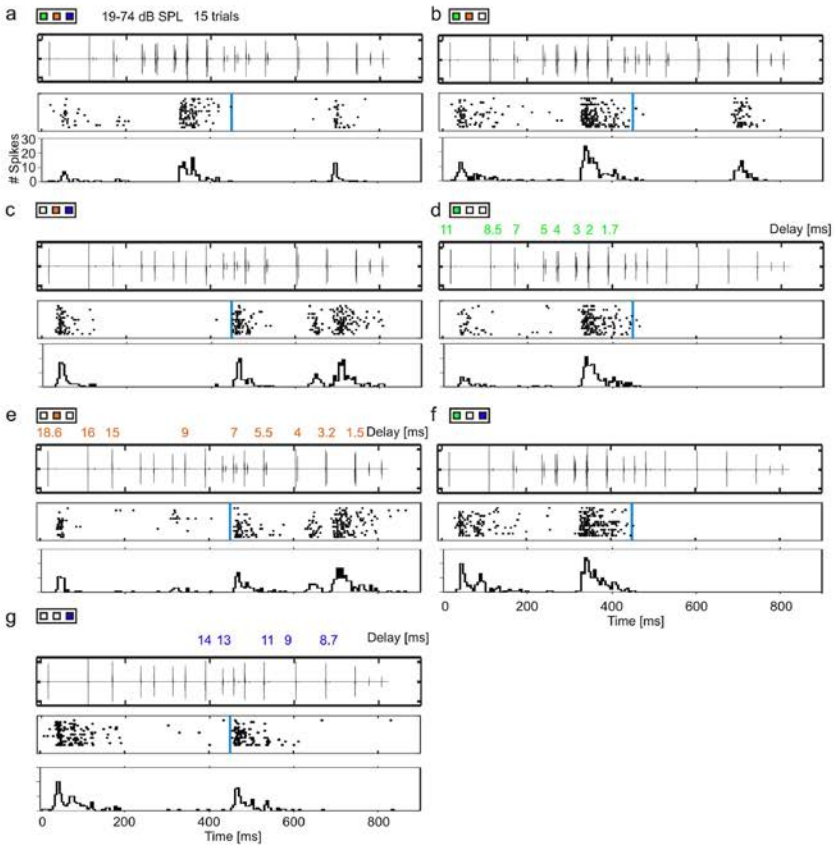

Figure 2. Responses to multiple- and to different single- and dual-object sequences. For each stimulus condition the oscillogram of the acoustic sequence (top plot), the raster plot, and post-stimulus time histogram (PSTH; binsize = 5 ms) of the neuronal response from a multi-unit is shown (middle and bottom plots). Vertical blue lines indicate the time point where echoes from object A are absent due to the bat leaving that object behind in the flight trajectory. Colored numbers above the oscillograms in (**d**,**e**,**g**) indicate some delays that are presented at that particular time point of the corresponding sequence. (**a**–**g**) Response to object ABC (**a**), AB (**b**), BC (**c**), A (**d**), B (**e**), AC (**f**), and C (**g**) sequences. Note that after passing object A, stimulations in (**a**,**c**,**b**,**e**) as well as stimulations in (**f**,**g**) are the same.

hampers the representation of echoes from more distant objects. Such suppression operates in the time window from 450 ms until the end of the sequence.

Forward suppression and recovery in response to multiple-object sequences. For the purpose of simplicity, the analysis of suppression described below is based on dual-object sequences. For getting an overview about the suppression in the multiple-object sequence see Supplementary Figure 2. We focussed here on the suppression in the dual-object sequence because responses to such sequences can be directly compared with responses to a single-object sequence where only echo information from one object is processed. Comparing responses to the multiple-object sequence with responses to dual-object sequences could be ambiguous because in dual-object sequences suppression could be caused by the presence of echoes from two objects.

To visualize the impact of object A on the neuronal tuning, the post-stimulus time histogram (PSTH) to the object B sequence was subtracted from the PSTH to the dual-object sequence containing echo information from object A and B (Fig. 3a). The obtained values were exemplarily plotted as activation rates into a line plot for one unit (Fig. 3b, *top*) but could also be transferred and plotted into a color-map (Fig. 3b, *bottom*). Note that bins showing no difference between the two PSTHs are indicated as white bins in the colormaps. Negative values indicate suppressive events while positive values indicate excitatory effects of object A on the response to the AB sequence. For getting an overview of the impact of object A on the neuronal tuning in each recorded unit, the

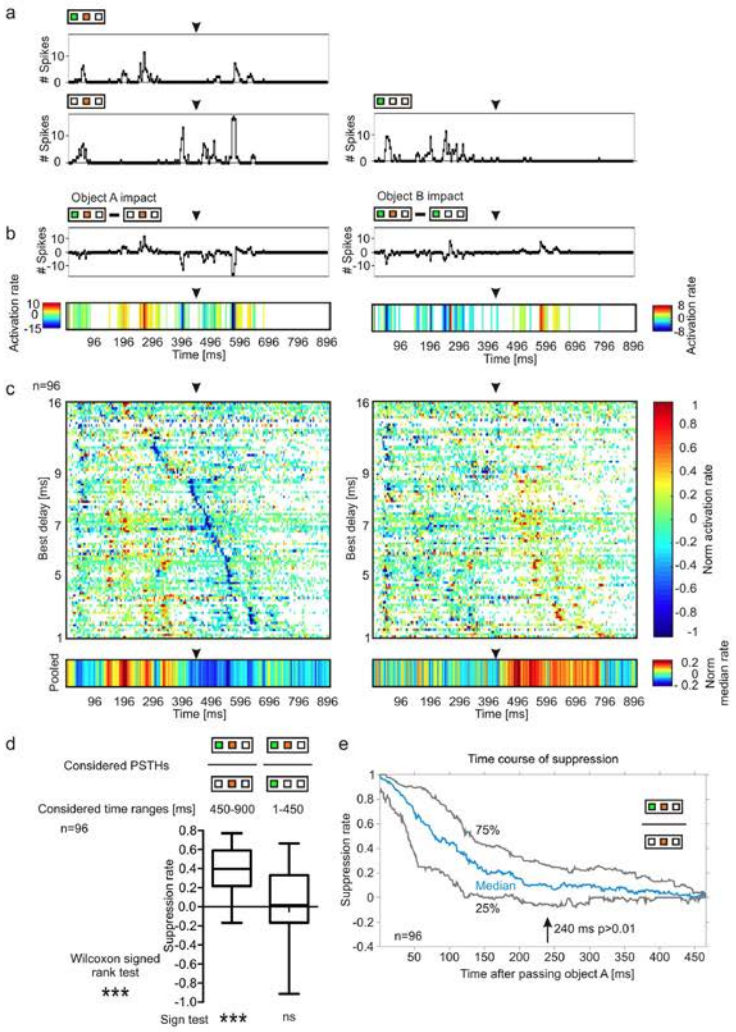

Figure 3. Quantification and time course of suppression when stimulated with a dual-object sequence. (**a**) Top: PSTH in response to the AB sequence. Bottom: PSTHs in response to the B and A sequence. Black arrowheads signal time point of passing object A. (**b**) Left: To investigate the impact of object A on the response to the AB sequence the PSTH to the B sequence was subtracted from the PSTH to the AB sequence. The obtained activation rates are plotted into a line plot and into a color-map. For the color-maps, bins with no difference between dual- and single-object sequences are white. Negative values indicate suppressive and positive values excitatory impacts of the corresponding object on the response to the dual-object sequence. Right: To investigate the impact of object B on the response to the AB sequence, the PSTH to the A sequence was subtracted from the PSTH to the AB sequence. The obtained activation rates were plotted as for the left subfigures. (**c**) Top: Color-maps of normalized activation rates from 96 units vertically ordered according to their best delays in response to the B sequence. Bottom: Pooled activation rates from all units. (**d**) Suppression rates calculated from responses to dual- and single-object sequences at specific time ranges. The response to the second half of the sequence (450 ms – end of the sequence) is suppressed when echoes from object A are present (sign test < 0.001; left boxplot). The presence of object B echoes has no effect on the response to object A when considering the time range where object A is presented (start – 450 ms; right boxplot; sign test: $p > 0.05$). The whiskers of the boxplots represent 5–95% percentile. (**e**) Time course of and recovery from suppression calculated with the normalized suppression rates from each unit in a bin-wise manner. Recovery occurred 240 ms after passing object A and it is defined as soon as the suppression rates did not differ significantly from 0 (sign test: $p > 0.01$ = no suppression).

normalized PSTHs were analysed and the normalized activation rates were plotted into a color-map (Fig. 3c, *left color-map*). Each unit is represented in one line and they are vertically ordered according to their delay tuning from 16 ms to 1 ms. For pooling the data, the median of the normalized activation rates from all units were plotted in a bin-wise manner at the bottom of Fig. 3c. Excitatory effects of object A on the response to the AB sequence were widely spread from the start until the 450 ms mark (black arrowheads in Fig. 3b,c). This is not surprising because in that time window echo information from object A was present in the AB sequence and processed by the units. Suppressive effects were prominent directly after the excitatory responses to object A. The time point of the suppression depended on the delay tuning of the units and occurred later in short- than in long-delay tuned units. For quantifying the suppression strength induced by object A, suppression rates were calculated by taking the ratio between the total amount of spikes in response to the AB sequence and the B sequence in the time window starting at the 450 ms mark. The obtained ratio was subtracted from 1 (Fig. 3d, *left boxplot*). The result of this calculation showed that in 89% (85 out of 96) of the units the response to object B was suppressed by the presence of leading echoes from object A and showed suppression rates above 0. The median suppression rate was significantly higher than 0 (median = 0.38; IQR = 0.22–0.58; sign test: $p < 0.001$).

To investigate the impact of object B on the response to the AB sequence, the normalized response to the A sequence was subtracted from the normalized response to the AB sequence and the values were plotted as a color-map (Fig. 3c, *right color map*). For an exemplary unit see Fig. 3b. Based on the pooled data, object B had no pronounced suppressive impact on the response to the AB sequence. The absence of suppression is reflected by the suppression rates calculated in the time window from the start to the 450 ms mark (Fig. 3d, *right boxplot*). The suppression rates did not deviate significantly from 0 (median = 0.02; IQR = −0.17–0.33; sign test: $p > 0.05$), which indicates that echoes from B had no impact on the neuronal response to object A. After the 450 ms mark, excitatory effects induced by the presentation of object B echoes were detected (Fig. 3c, *right color-map*). Note that echoes from object B at that time window were absent in the object A sequence.

Next, we wanted to assess the time course of and recovery from suppression that is induced by object A. The number of spikes from the 450 ms mark until the end of the sequence in response to the object B sequence was subtracted from the spike count evoked by the AB sequence in a bin-wise manner. For each unit, the resulting spike-count differences obtained for each bin were normalized to the maximum absolute difference and averaged to create a pooled suppression rate for each 5 ms bin (Fig. 3e). The results of this calculation showed that right after passing object A (time point 0 in Fig. 3e) the suppression was maximal, as indicated by suppression rates close to 1. The suppression decreased over time reaching values that were not significantly different from 0, starting at ~240 ms after passing object A (sign test: $p > 0.01$).

Impact of each object on the neuronal response. After characterizing the suppression, we wanted to quantify the relative impact of each object on the response pattern to the multiple-object sequence that contained echoes from the three objects. For each unit, the time point of the best response was calculated in response to the ABC sequence and to each of the single-object sequences. At the cortical level, delay-tuned neurons are topographically organized in some bat species, including *C. perspicillata*[8]. In the dorsal auditory cortex of *C. perspicillata*, neurons tuned to short delays are confined to rostral regions while neurons tuned to long delays appear mostly in caudal regions. A representative response pattern from six units recorded simultaneously with an electrode array positioned along the chronotopic gradient of the dorsal auditory cortex of *C. perspicillata* is shown in Fig. 4. Each row of the color-maps represents one unit recorded at a specific cortical location (denoted by dot color; Fig. 4a). Note that the *orange* unit in Fig. 4 is the example unit shown in Fig. 3a,b. According to the neuronal activity in response to the B sequence (Fig. 4d), the caudal units (unit 6, unit 7, unit 8, and unit 11) had best delays in the range of 7–9 ms, whereas the two remaining rostral units, unit 10 and unit 9 had best delays between 4 and 5 ms. When comparing the neuronal response to the multiple-object sequence (Fig. 4b) with the response to the single-object sequences (Fig. 4c–e), it is evident that the time points of the best responses (*white dots* in Fig. 4) in the object A sequence are close in time to the best responses observed in the multiple-object sequence. In contrast, the time points of best responses in the two remaining single-object sequences differ vastly from the time points of best responses in the multiple-object sequence.

The same results can also be seen in the pooled data (Fig. 4f). Differences of best responses (response shifts) are significantly smaller between the multiple-object sequence and object A sequence than between the multiple-object sequence and the B or the C sequences. Based on these observations one could propose that the response to the multiple-object sequence is strongly shaped by echoes from object A. The same results can also be seen for processing the dual-object sequences (Supplementary Fig. 3).

To take the overall activity pattern into account, the PSTH obtained in response to the multiple-object sequence was correlated with the PSTHs to each single-object sequence. The higher the correlation indices (CIs), the more similar are the compared PSTHs. In the example units from Fig. 4, and at the population level (Fig. 5a) the CIs between the single-object sequences and the multiple-object sequence were maximal when comparing the response to the multiple-object sequence with the response to the A sequence (see CI values at the right corner of Fig. 4c–e). Thus, the PSTHs to the A sequence are the ones that resemble more the PSTH obtained in responses to the multiple-object sequence. These results suggest that in a multi-echo environment, responses are mostly determined by the first-arriving echo, in this case the echo from object A, while the responses to later echoes are either absent or partly suppressed. This idea is further confirmed by the fact that before passing object A (start of sequence until 450 ms mark) CIs were highest between PSTHs obtained in response to the A sequence and PSTHs obtained when playing the ABC sequence (Fig. 5b). After passing object A (after 450 ms mark until the end of the sequence), a situation in which the echoes from object B are leading, the response to the B sequence led to relatively high CIs while the CIs obtained from the C sequence were similar to those obtained from the A sequence, even though echoes from object A were absent after 450 ms into the sequence (Fig. 5c). Note that, at the population level, the neuronal response to the multi-object sequence was more similar to the response to the dual-object

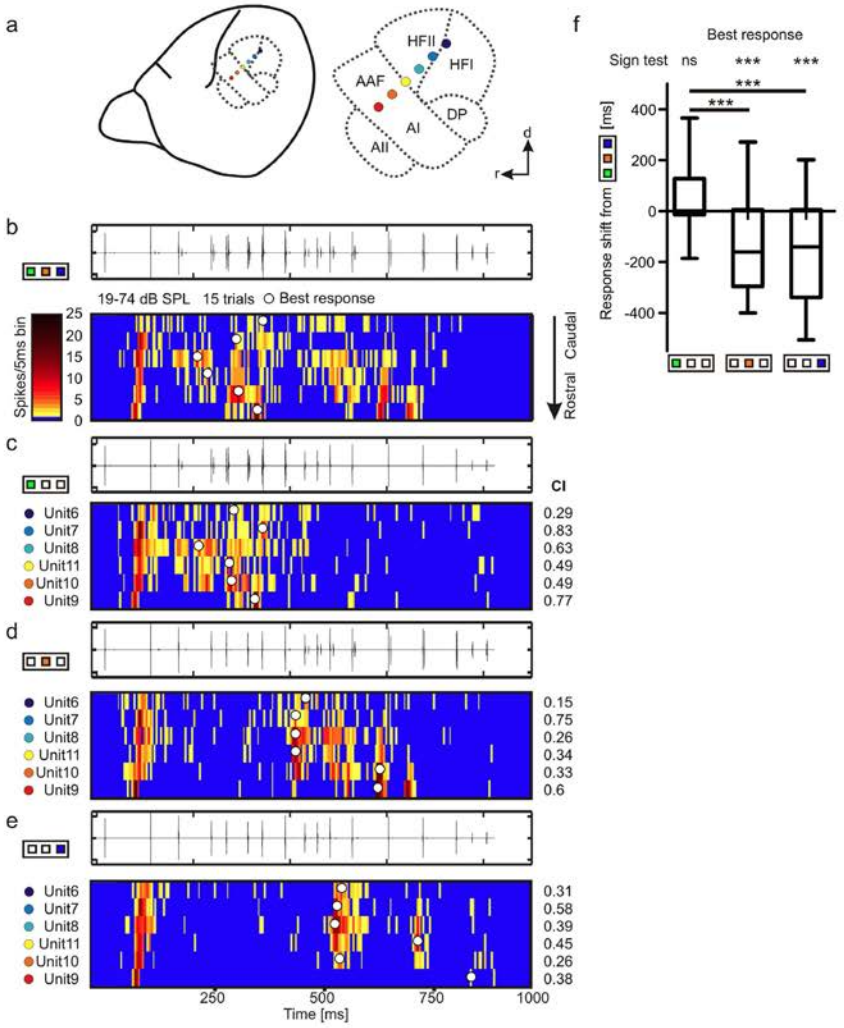

Figure 4. Responses to multiple- and single-object sequences of six units recorded simultaneously within the chronotopically organized cortex regions. (**a**) Schematic lateral view on *C. perspicillata*'s brain and magnified auditory cortical areas (dashed lines). Colored spots denote electrode positions. The linear electrode array was positioned slightly oblique along the rostro-caudal axis and covered part of the high frequency areas end extended into the high frequency regions of the primary auditory cortex. d = dorsal, r = rostral. (**b**–**e**) For each stimulus the oscillogram (upper) and activity pattern of six units in response to the ABC (**b**), A (**c**), B (**d**), and C (**e**) sequences are shown in a color-map with a binsize of 5 ms. Each row represents the activity pattern from one unit in response to 15 trials. The units were recorded simultaneously from the auditory cortex and their positions follow the chronotopy along the caudo-rostral axis of the cortex (**a**). White dots in color-map mark the time point of best responses. The correlation indices (CIs) are indicated at the right side. In five out of six units, CIs were higher between the response pattern to the A sequence and the multiple-object sequence than between responses to the B or C sequences and the multiple-object sequence. (**f**) Response shifts for the best response mediated by each object are plotted against each other. Respectively, positive or negative shifts indicate that the response occurs later or earlier in the multiple-object sequence than in the corresponding single-object sequence. Best response differed minimally between multiple-object sequence and the A sequence. Best response differed more strongly between multiple-object sequence and the B or C sequences. Thus, the delay tuning in response to the multiple-object sequence is mostly determined by object A and less by object B and C. Kruskal-Wallis and Dunn's multiple comparison post hoc test (***$p < 0.001$).

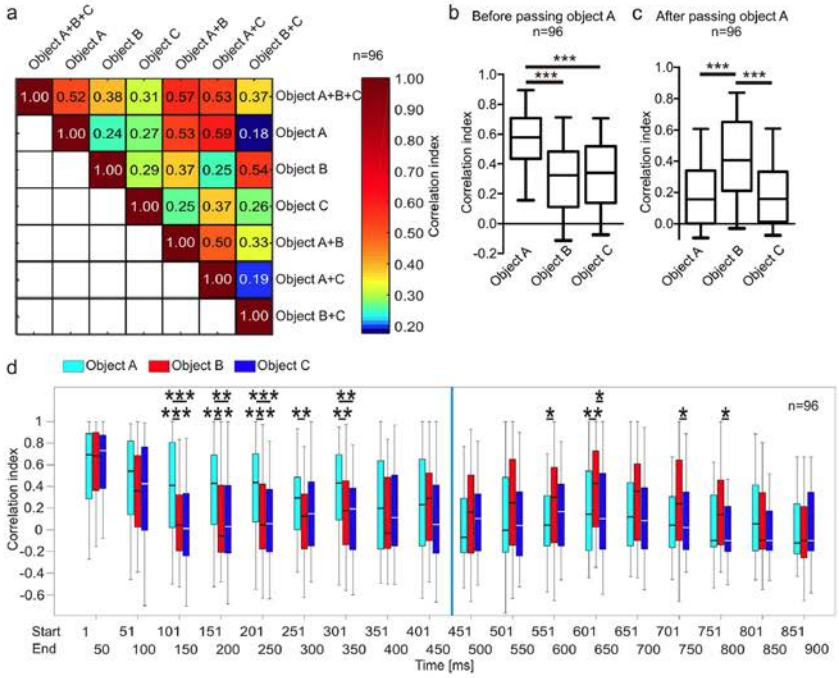

Figure 5. Neurons focus on spatially closest objects. (**a**) Pooled correlation indices from PSTHs in response to multiple-, dual- or single-object sequences. (**b**) Correlation indices between PSTHs of each single-object sequence and to the multiple-object sequence before passing object A. (**c**) Correlation indices between PSTHs to each single-object sequence and to the multiple-object sequence after passing object A. (**d**) Time course of correlation indices calculated from 50 ms time windows of the PSTHs of each single-object sequence correlated to the corresponding time windows in the multiple-object sequence. Each 50 ms time window consisted of ten 5 ms bins of the PSTHs. Note that before passing object A the PSTH in response to the A sequence mostly resembles and thus has highest impact on the response pattern to the multiple-object sequence (indicated by significantly higher correlation indices for *cyan* boxplots). After passing object A the PSTHs in response to the B sequence mostly resembles the PSTH in response to the multiple-object sequence (indicated by significantly higher correlation indices for *red* boxplots; Kruskal-Walis and Dunn's multiple comparison post hoc test; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

sequences containing echo information from object A (CIs = 0.57 and 0.53, respectively) than to any response to the single-object sequence (CIs = 0.52; 0.38 and 0.31; Fig. 5a). This result reflects the physical similarity between the acoustic stimuli. The dual-object sequences contain more echoes from the multiple-object sequence than the single-object sequences, and the latter is reflected in the response patterns.

In a more detailed temporal analysis, the CIs were calculated with 50 ms bins where ten 5 ms bins from the PSTHs to the ABC sequence were compared with the corresponding bins from the PSTHs to the single-object sequences. Here, at the beginning of the stimulation, the CIs were relatively high for each object but after 100 ms it becomes evident that object A strongly drives the activity pattern in response to the ABC sequence (Fig. 5d). The high CIs at the beginning of the sequence can be explained based on strong initial responses to acoustic stimuli a phenomena that has already been described in a previous study[14]. From 400 ms on (directly before object A is passed), the CIs from object B increased until being significantly higher than the CIs from the A or C sequence at the time windows 551–600 ms. It could be that at that time point, enough neurons had already recovered from the suppression caused by object A, and thus the response to the object B sequence resembles best the response to the multiple-object sequence. Note that the impact of object C was relatively low throughout the stimulation in the multiple-object sequence, which could point towards a forward suppression that was induced by object B after the 450 ms mark.

Discussion

In their natural environments, animals have to cope with multiple stimuli arriving simultaneously or sequentially to their sensory organs. Not each stimulus has the same behavioral relevance. Therefore, it would be expected that stimuli are processed differentially in the brain depending on their ethological value. Studies in vertebrates and

invertebrates have revealed that animals focus their visual gaze in a complex scenario and that they change their focus from time to time through saccadic movements[2,18–24]. Behavioral experiments have already demonstrated that echolocating animals, like bats and toothed whales, fixate the acoustic gaze through spatially focusing their sonar beam[6,10,25–33]. The latter has been interpreted as a mechanism that attempts to reduce the amount of echo reflections produced by surrounding objects. A recent study in the species *Phyllostomus discolor*, a close relative to *C. perspicillata*, showed that the spatial focus of the sonar beam seems to be extremely dynamic and the restriction is facultative for the animals[34]. It appears that in bats spatial restriction of the sonar beam is not mandatory for proper orientation. Therefore, it is unlikely that in target-rich environments only one echo is produced per biosonar call emission, even if the bats restrict their sonar beam. Note that the multiple echoes derived from each call emission are not overlapping in time, and therefore they do not create spectral notches that can be used for µs time estimations, as describe for *Eptesicus fuscus*[35,36]. We discuss three scenarios on how echo information from multiple objects can be processed in the brain of echolocating bats. (i) Echo information from all objects is processed in multiple streams in the cortex (parallel processing). Theoretically, if the distance between two objects is long enough, then short delay-tuned neurons could process the target distance from the nearest object, whereas echoes from more distant objects could be processed by long-delay tuned neurons. (ii) Echo information from one object is preferentially processed (single object processing). (iii) Not the distance between the animal and the object is processed, but the distance between multiple objects is processed with the help of the delay between consecutive echoes. Note that processing the distances between objects would be less beneficial to the animal, assuming that the bat's main goal is to determine target distance to avoid collisions. However, it may have advantage for complex scene analysis.

Delay tuning depends on an interaction between excitation and inhibition[11,37]. The broadcasted call usually evokes an inhibition followed by a rebound excitation. If rebound excitation triggered by the call coincides with an excitation in response to the echo, then firing occurs in the delay tuned neurons. Therefore, a preceding call opens a temporal integration window for processing echo information. Because of the weakened response to second or third echoes described in the present study, one could suggest that the most sensitive temporal processing window is closed after first echo arrival.

A processing strategy based on parallel processing (Hypothesis 1) provides the animal with the most detailed distance information about its surrounding. However, this coding strategy may not be ideal for initiating fast motor responses because the cortex has to cope simultaneously with multiple processing streams. A coding strategy that focuses on distance information from one specific object would be simpler, and our results show that cortical responses to multiple-object sequences are essentially determined by responses to leading echoes. Responses to lagging echoes are subjected to suppression. Thus, there seems to be a "default" preference in the neuronal tuning to close objects by cortical units that is robust and evident even in anaesthetised animals. In other words, cortical suppression does not only increase the sharpness of delay-tuning as demonstrated in previous studies[14,38–40] but it might also help the animal to focus by default on near obstacles while flying in multi-object environments. Parallel processing and processing distances between objects are unlikely to be realized without selective attention that may be used to allow a correct identification of an auditory event to be a call or an echo. Without such processing taking place, cortical neurons appear to define by default the first auditory event as a call and the second event as an echo. Echoes occurring shortly afterwards are largely subjected to cortical suppression, which also prevents a misdefinition of call and echo.

The presence of cortical suppression avoids parallel processing of multiple auditory streams. A parallel processing strategy has been proposed by electrophysiological studies that used simple single call/multiple echo elements[39,41]. Suppressing distance information from lagging echoes occurs at the expense of losing information from distant targets. However, one has to keep in mind that this suppression could be of advantage for the generation of fixed motor patterns important for rapidly moving animals that, like bats, have to orientate in permanently changing and complex environments. In other words, focussing cortical neuronal responses by default on the nearest object could help the animal to avoid crashing into an immediate obstacle during flight. This does not mean that during echolocation, echoes from distant objects are not processed. Behavioral studies showed that after cortical ablation, bats are still capable to avoid collisions during flight[42]. A phenomena that is abolished after bilateral ablation of ventral parts of the inferior colliculus[43]. How subcortical structures like the inferior colliculus process the echolocation streams of the present study should be addressed in future studies. Additionally, for long range orientation bats could rely on vision and memory for planning their flight path[44–46]. This memory could be adjusted through delay information coming from near objects. Finally selective attention could additionally affect neuronal tuning by acting directly on the neurons or by changing the amount of information that reaches the cochlea via the efferent system[47,48] or changes in pinna position and/or in the stiffness of the middle ear[49]. Different behavioral adjustments can change the sensory world that bats are facing or perceiving. During call emission, the animals can variably adjust their sonar beam[49] and the echo perception can be influenced through motor behaviors of the head or the pinnae[49,50]. A recent study on insect-eating bats demonstrated that, while hunting, the bats shift their sonar beam and flight path towards the second prey before capturing the immediate prey which increases the capture rate[51]. In the present study, the effects of attention could not be tested because neuronal recordings were performed in anesthetized bats. However, it is possible that the attention of the animal that was swinging in the pendulum for stimulus recordings could be represented on the call parameters of the echolocation sequence. It is known that other bat species like *E. fuscus* actively adjust their sonar parameters when in multiple object environments to focus on objects that are far away[6,10]. Such behaviour has not been demonstrated in fruit eating bats, such as the one studied here, and therefore whether and how it influences neuronal responses remains, at present, unknown.

In conclusion which impact top-down mechanisms like attention or behavioural state of the animal has on the coding strategy remains speculative but it is noteworthy, that even without selective attention the animal could process target-distance information from the nearest obstacle.

Methods

Animals. Electrophysiological experiments were conducted in six adult bats (5 females and 1 male) of *Carollia perspicillata*. The bats were bred in a colony of the Institute for Cell Biology and Neuroscience (Frankfurt University). The animal use in the experiments complies with all current German laws on animal experimentation and it is in accordance with the Declaration of Helsinki. All experimental protocols were approved by the Regierungspräsidium Darmstadt (experimental permit #F104/57).

Stimulus recordings, constructions and presentations. For recording a natural echolocation sequence that was used later on as acoustic stimulus on anesthetized bats, a bat was placed in a pendulum[12–14]. An ultrasound sensitive microphone (Avisoft Bioacoustics, Germany) was medially positioned above the animal's head and adjusted as close as possible to the ears (~4 cm). The microphone had a sensitivity of 50 mV/Pa and an input-referred self-noise level of 18 dB SPL. It was connected with a sound acquisition system (UltraSoundGate 116Hm mobile recording interface, +Recorder Software, Avisoft Bioacoustics, Germany) for sound digitalization at 375 kHz (16 bit precision). The bat was swung (total distance = 4 m) and it faced three objects that were separated along the depth axis during the swing. Object A was a dummy rock (depth: 65 cm; width: 95 cm; height: 35 cm) made out of papier-mâché and it was overflown by the animal before the swing stopped in front of object B, a wooden plate (depth: 0.8 cm; width: 21 cm; height: 21 cm), which was positioned 130 cm after object A. 20 cm behind object B was object C an acrylic glass wall (depth: 0.3 cm; width: 50 cm; height: 150 cm). During the swing the animal broadcasted sequences of calls which were recorded. The acrylic glass wall covered a larger area than the wooden plate, thus echoes coming from both objects were recorded.

Background noise of the stimuli was filtered via "Noise Reduction" (FFT length 256; precision 16) with the software Avisoft SAS Lab Pro (Avisoft Bioacoustics, Germany) as described in ref. 14. To transform the multiple- into single- and dual-object sequences we manually filtered with the software BatSound (PetterssonElektronik AB, Sweden) the echoes belonging to specific objects.

During electrophysiological recordings, acoustic stimuli were played at a sampling rate of 384 kHz (32 bit precision) with an Exasound E18 sound card (ExaSound Audio Design, Canada). The audio signals were transferred to an audio amplifier (Rotel power amplifier, RB-850). The bat was stimulated with a calibrated speaker (ScanSpeakRevelator R2904/7000, Avisoft Bioacoustics, Germany) located at 15 cm from the bat's ear. The calibration curve was calculated with a ¼-inch Microphone (Brüel&Kjaer, model 4135, Denmark) which was connected to a custom-made microphone amplifier.

The echolocation sequences were played randomly with 15 averages and at intervals of 400 ms. Three different intensity levels were used. The maximal intensity level ranged between 29–84 dB SPL (Supplementary Table 1) and the sequences were attenuated 10 dB and 20 dB for the remaining two sound pressure levels.

Data acquisition and analysis. Electrophysiological recordings took place in a sound-proofed and electrically-shielded chamber. Neuronal responses were recorded from both brain hemispheres. For anaesthesia, bats were subcutaneously injected with a mixture of ketamine (10 mg × kg^{-1} Ketavet, Pharmacia GmbH, Germany) and xylazine (38 mg × kg^{-1} Rompun, Bayer Vital GmbH, Germany). Surgery and chronical recordings were done as described in ref. 14.

Recordings were performed with custom-built glass electrode arrays of up to 6 channels organized in a row. Glass electrodes (resistance 1–10 MΩ when filled with 3 Mol KCl) were drawn from borosilicate capillaries (GB120F-10, Science Products, Germany) with a Flaming/Brown horizontal puller (P97, Sutter, USA) and they were glued together in a fanshape pattern, thus ensuring an electrode tip space of 250 µm. Penetration occurred with the dura mater remaining intact. The glass electrode arrays were positioned in the high frequency area of the auditory cortex[52] along the chronotopic gradient[15]. The high frequency area was located caudal to a blood vessel within the depression of the pseudocentral sulcus[15]. Along the dorso-ventral axis the electrodes were positioned 1–2 mm lateral from the scalp midline. The orientation of the electrode array was adjusted in parallel to the scalp midline. Based on frequency-level receptive fields, all analysed multi-units were sensitive to high frequencies. Frequency tuning was assessed with pure tone stimuli of 2 or 10 ms duration (0.5 ms rise-fall time). Tested frequencies ranged from 5–95 kHz and the sound pressure levels were between 30–90 dB SPL. Sound levels were adjusted based on the speaker's calibration curve. Each frequency-level combination was randomly presented five times with a 400 ms interstimulus time interval. All multi-units sensitive to high frequencies were delay-tuned. Delay-tuning was assessed with a stimulus protocol that allowed a rough calculation of a delay-tuning curve as described in ref. 14.

Neuronal data acquisition used a wireless multichannel recording system (Multi Channel Systems MCS GmbH, Germany), at a sampling rate of 20 kHz (per channel) and 16 bit precision. Neuronal responses were analysed in 96 multi-units. Spike events were detected with a multi-unit specific threshold that was based on the spike amplitude. For each multi-unit, spike threshold was kept constant during the stimulation protocol thus ensuring that the same multi-unit activity was recorded for each stimulus.

All analyses are based on post-stimulus time histograms (PSTHs) used a binsize of 5 ms. Sign tests were calculated in Matlab 2014 and remaining statistics in GraphPad Prism 5 (GraphPad Software, USA). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Only the best intensity for each unit represented by the highest number of evoked spikes in response to the multiple-object sequence was analysed. The time window for best response calculations were from 150 ms to the end of the sequence. The first 150 ms were not considered because strong initial responses often occurred which were not related to delay tuning. To test the similarity between the PSTHs calculated based on the neuronal responses to the multi-, dual- and single-object sequences a correlation analysis was done in Matlab 2014. The higher the similarity, the higher the obtained correlation indices. Correlation calculations were done within different time windows of the PSTHs. For rough overviews a correlation was done through taking the entire PSTH length into account. For detailed correlation analysis the first 450 ms of the PSTHs (before passing

object A), after the 450 ms mark (after passing object A) or in 50 ms bins were analysed. The analysis in 50 ms time windows took ten 5 ms bins of the PSTHs into account for the calculation of correlation indices.

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Author Contributions

M.J.B. performed experiments. J.C.H. wrote scripts for recordings. J.C.H. and M.J.B. wrote scripts for analysis. M.J.B. analysed data. M.J.B. wrote manuscript. M.J.B., J.C.H. and M.K. conceived and directed the study. All authors discussed the results and commented on the manuscript.

Additional Information

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Supplements

Table S1 Sequence paramters (refers to Fig. 1)

Calculations were done with Avisoft SASLab Pro (Avisoft Bioacoustics, Germany).
BW = bandwidth; dur = duration; duty cycle = call duration/call interval; f = frequency; int = intensity; rms = root mean square

Polyobject sequence														
# call	dur	Pulse interval	1st delay	2nd delay	3rd delay	int pulse	int 1st echo	int 2nd echo	int 3rd echo	duty cycle	peak f	min f	max f	BW
	[ms]	[ms]	[ms]	[ms]	[ms]	rms [dB SPL]	rms [dB SPL]	rms [dB SPL]	rms [dB SPL]	[%]	[kHz]	[kHz]	[kHz]	[kHz]
1	1.27		11	18.6		76.99	55	54		1.34	82.6	77.4	90.2	12.7
2	1.41	94.45	8.5	16		80.89	62	61		2.46	84.9	79.9	91	11.1
3	1.37	57.37	7.3	15		80.65	67	62		2.03	86.8	81.8	91.9	10.1
4	1.39	67.58	5	10.7		75.66	75	42.5		4.41	71.9	82.1	92.9	10.7
5	1.41	31.52	4	9.3		78.87	79	50.63		3.19	72.7	76.2	93.6	17.3
6	1.62	44.22	3	9.2		78.02	80	45.47		5.47	76.2	83.6	91.4	7.7
7	1.33	29.6	2.3	8.9		78.1	79.4	41.01		2.78	66	80.4	92.8	12.3
8	1.31	47.83	1.7	8.7	14.2	81.5	71.6	65.3	28.95	3.23	60.3	80.3	94.1	13.7
9	1.22	40.52		7.7	13	75.27		67.6	52.1	4.72	84.4	78.6	95	16.3
10	1.1	25.83		7	12.2	76		68.9	52.5	4.19	84.8	79.9	92.4	12.5
11	1.37	26.25		6.5	11.6	77.42		73.5	57.6	3.00	63.6	81.1	95.8	14.6
12	1.77	45.64		5.5	11	80.58		70.5	58.1	2.34	65.1	80	92.3	12.3
13	1.43	75.56		4	9	81.08		78.1	44.87	2.02	86.9	84.2	94	9.8
14	1.33	70.79		3.2	8.7	80.31		83.3	56.98	1.92	83.8	73	93	20
15	1.06	69.43		1.5		82.33		83.7		3.22	83.8	74.7	94.3	19.5
16	0.77	32.91		1.3		72.8		74.5		2.59	79.2	75.8	91.6	15.7
17	0.81	29.75		1		75.68		76.2			76.5	70	92.8	22.7

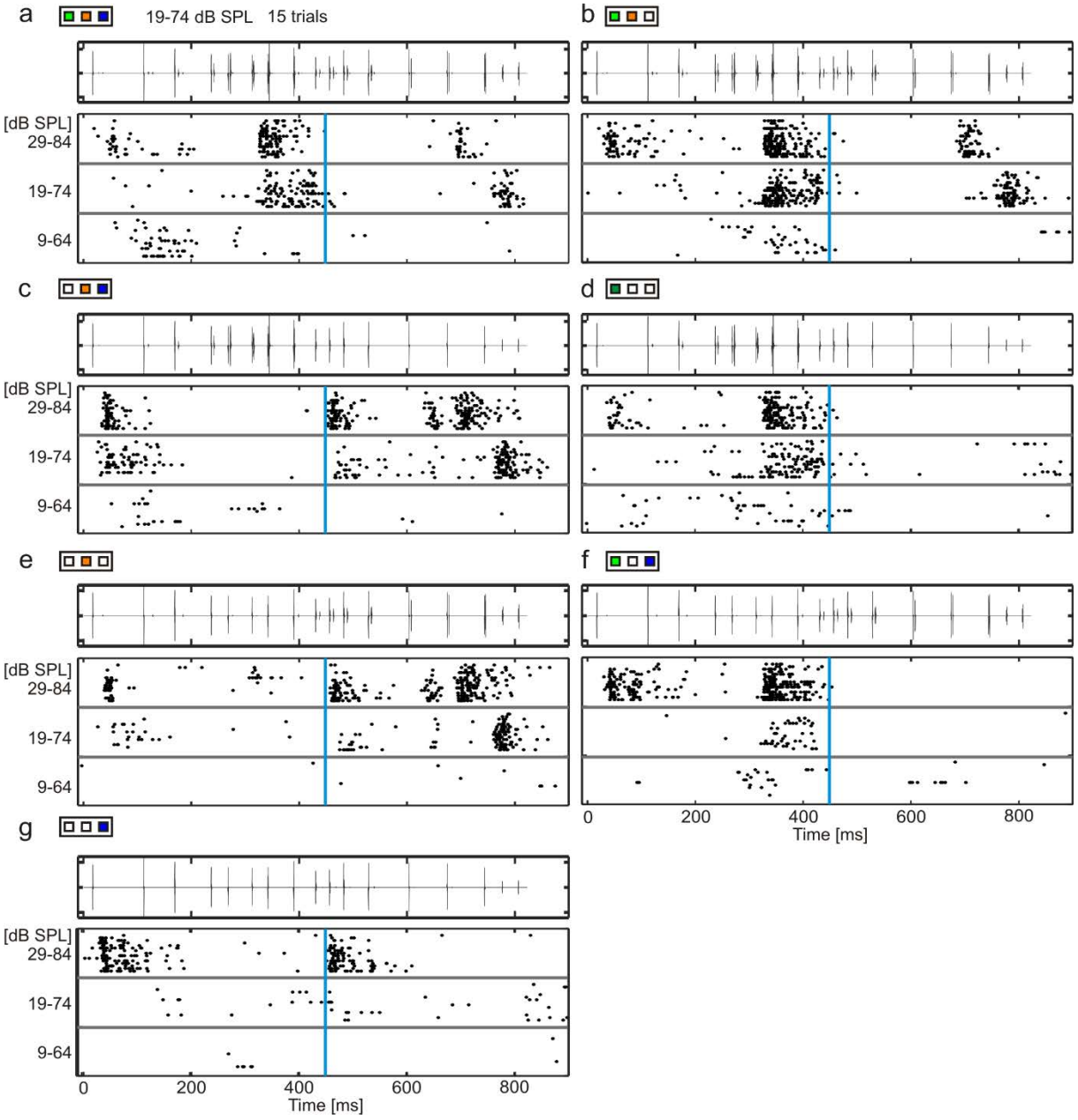

Figure S1. Responses of one unit to different single- and dual- and multiple-object sequences played at three different attenuation levels (refers to figure 2).

For each stimulus the oscillogramm (upper) and raster plot in response to all three attenuation levels are shown. Vertical blue line indicate the time point where echoes from object A disappear due to the bat leaving that object behind in the flight trajectory.

(a-g) Response to object ABC (a), .AB (b), BC (c), A (d), B (e), AC (f) and C (g) sequence. Note that echolocation sequences (b-g) were created through filtering manually the corresponding echoes from sequence (a).

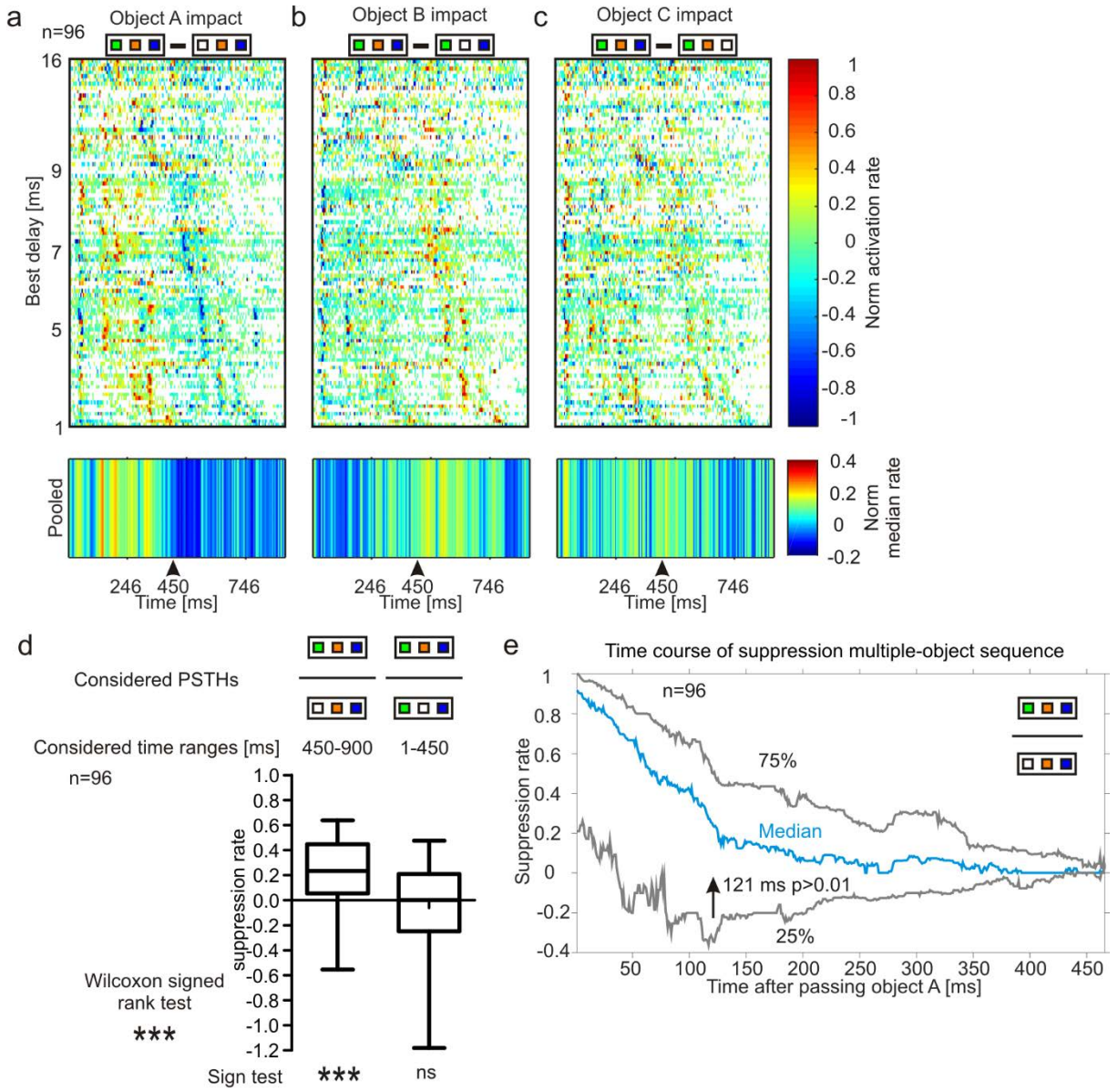
a
n=96
Object A impact
b
Object B impact
c
Object C impact
Best delay [ms]
16
9
7
5
1
Norm activation rate
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Pooled
Norm median rate
0.4
0.2
0
-0.2
246 450 746
Time [ms]
d
Considered PSTHs
Considered time ranges [ms]
450-900
1-450
n=96
suppression rate
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
Wilcoxon signed rank test
***
Sign test
***
ns
e
Time course of suppression multiple-object sequence
n=96
75%
Median
121 ms p>0.01
25%
Suppression rate
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
50 100 150 200 250 300 350 400 450
Time after passing object A [ms]

Figure S2. Quantification and time course of suppression when stimulated with the ABC sequence (refers to figure 3).
(a-c) Top: Color maps of normalized activation rates from 96 units vertically ordered according to their best delays in response to the B sequence. Activation rates were calculated through subtracting the PSTHs in response to the BC (a), AC (b) and to the AB (c) sequences from the PSTHs in response to the ABC sequence. Bins, with no difference between the response to the ABC sequence and to the dual-object sequence are white. The activation rates show the relative impact of each object to the overall response along the temporal axis. Negative values indicate suppressive and positive values excitatory impacts of the corresponding object on the response to the ABC sequence. Bottom: Normalized median activation rates from all units. Black arrowheads signal time point of passing object A. Note that after passing object A the response to object B is suppressed in response to the ABC sequence in comparison to the response to the BC sequence (a). Object B and object C had respectively, slight or no suppressive impact on the response to the ABC sequence (b and c). (d) Suppression rates calculated from responses to the ABC sequence and to the dual-object sequences under consideration of specific time windows. Response to object A in the first time window (1-450 ms) is followed by suppression in the second time window (450-900 ms; signtest < 0.001; left boxplot). The presence of object B echoes had no effect on the response to object A in the first time window (right boxplot; sign test: ns). (e) Time course of suppression and recovery range calculated with the normalized suppression rates from each unit and bin for the three object situation. Recovery occurred as soon as the values did not differed significantly from 0 (sign test: $p > 0.01$ = no suppression) and is indicated by a black arrow.

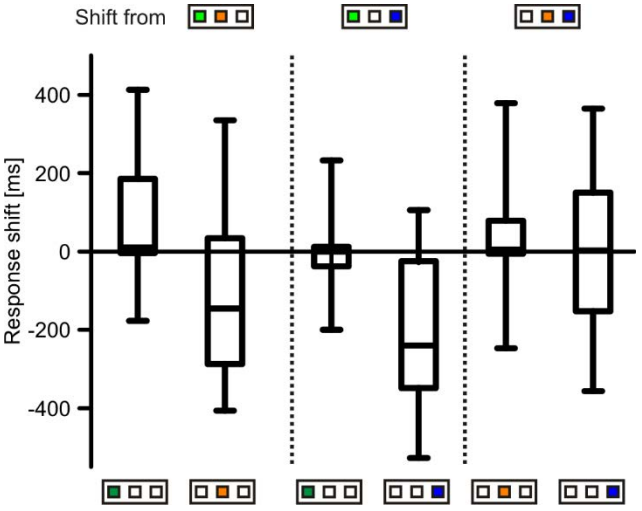

Figure S3. Shifts in time points of best responses induced by cortical suppression in the dual-object sequence (refers to Fig. 4).
Best response shifts between the dual- and single-object sequences. Note that in comparison to the data from figure 4, the response shifts are less between the leading echo and the dual-object sequence, indicated by response shifts that are closer to 0 and smaller quartile ranges than between the lagging echo and the dual-object sequence.

Processing of natural echolocation sequences in the inferior colliculus of Seba's fruit eating bat, *Carollia perspicillata*

M. Jerome Beetz, Sebastian Kordes, Francisco García-Rosales, Manfred Kössl, Julio C. Hechavarría

Status under revision

Abstract For the purpose of orientation, echolocating bats emit highly repetitive and spatially directed sonar calls. Echoes arising from call reflections are used to create an acoustic image of the environment. The inferior colliculus (IC) represents an important auditory stage for initial processing of echolocation signals. The present study addresses the following questions: i) How does the temporal context of an echolocation sequence mimicking an approach flight of an animal affect neuronal processing of distance information to echo delays? ii) How does the IC process complex echolocation sequences containing echo information from multiple objects (multi-object sequence)? Here we conducted neurophysiological recordings from the IC of ketamine-anaesthetized bats of the species *Carollia perspicillata* and compared the results from the IC with the ones from the auditory cortex. Neuronal responses to an echolocation sequence was suppressed when compared to the responses to temporally isolated and randomized segments of the sequence. The neuronal suppression was weaker in the IC than in the AC. In contrast to the cortex, the time course of the acoustic events is reflected by IC activity. In the IC, suppression sharpens the neuronal tuning to specific call-echo elements and increases the signal-to-noise ratio in the units' responses. When presenting multiple-object sequences, despite collicular suppression, the neurons responded to each object-specific echo. The latter allows parallel processing of multiple echolocation streams at the IC level. Altogether, our data suggests that temporally-precise neuronal responses in the IC could allow fast and parallel processing of multiple acoustic streams.

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Manuscript title: Processing of natural echolocation sequences in the inferior colliculus of Seba's fruit eating bat, *Carollia perspicillata*

Abbreviated title: Processing of natural stimuli in the bat midbrain

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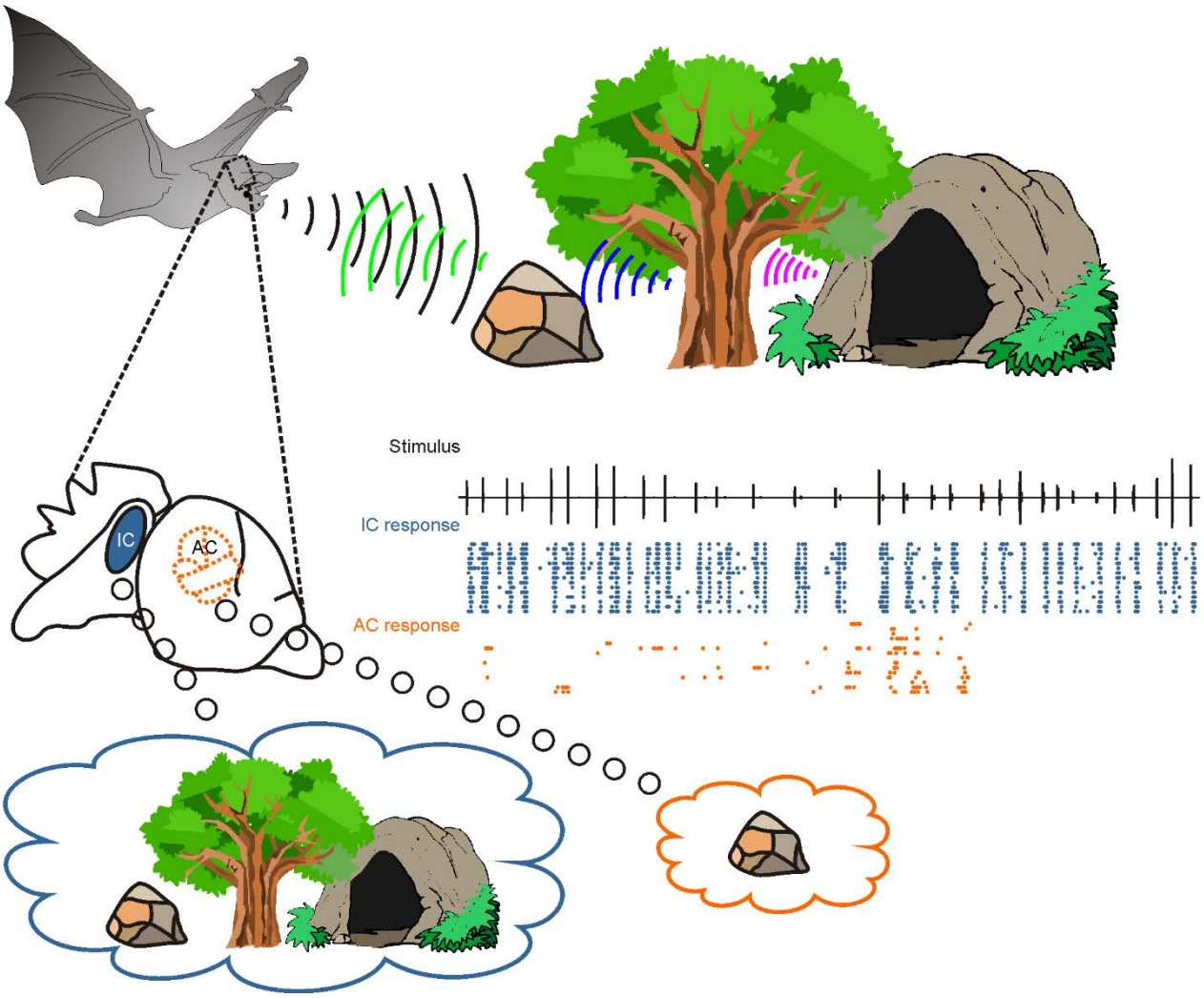
Visual Abstract



Abstract

For the purpose of orientation, echolocating bats emit highly repetitive and spatially directed sonar calls. Echoes arising from call reflections are used to create an acoustic image of the environment. The inferior colliculus (IC) represents an important auditory stage for initial processing of echolocation signals. The present study addresses the following questions: i) How does the temporal context of an echolocation sequence mimicking an approach flight of an animal affect neuronal processing of distance information to echo delays? ii) How does the IC process complex echolocation sequences containing echo information from multiple objects (multi-object sequence)? Here we conducted neurophysiological recordings from the IC of ketamine-anaesthetized bats of the species *Carollia perspicillata* and compared the results from the IC with the ones from the auditory cortex. Neuronal responses to an echolocation sequence was suppressed when compared to the responses to temporally isolated and randomized segments of the sequence. The neuronal suppression was weaker in the IC than in the AC. In contrast to the cortex, the time course of the acoustic events is reflected by IC activity. In the IC, suppression sharpens the neuronal tuning to specific call-echo elements and increases the signal-to-noise ratio in the units' responses. When presenting multiple-object sequences, despite collicular suppression, the neurons responded to each object-specific echo. The latter allows parallel processing of multiple echolocation streams at the IC level. Altogether, our data suggests that temporally-precise neuronal responses in the IC could allow fast and parallel processing of multiple acoustic streams.

Significance statement

High stimulus rates usually result in a reduction of neuronal responses that can be described as suppression or adaptation. It remains unclear how neuronal suppression influences sensory processing in animals that rely on high stimulus rates, as it is the case of bats. The present study investigates how natural echolocation sequences are processed in the bat's inferior colliculus. We report that collicular suppression enhances the signal-to-noise ratio of the spiking activity without degrading the temporal processing of echolocation sequences. Collicular suppression allows for a high tracking ability of the stimulus envelope and for the parallel processing of multiple auditory streams.

Introduction

The sensory world is dynamic and animals continuously receive sensory information from the environment. The temporal context, in which stimuli occur often carries behaviorally relevant information. Temporal parameters, like repetition rate, signal duration or inter-signal intervals, are utilized to identify conspecifics, a strategy that has been described in drosophila (Coen et al., 2014), crickets (Hedwig, 2006; Ronacher et al., 2015) and frogs (Feng et al., 1990; Gerhardt, 2005). Bats also rely on fast acoustic repetition rates for coping in everyday life scenarios. They orientate acoustically in the dark using echolocation by integrating high acoustic rates of call-echo information (Moss and Surlykke, 2010; Simmons, 2012; Kössl et al., 2015). Though fast acoustic repetition rates are important for many animal species, encoding these fast time-varying signals is challenged by the fact that repetitive stimuli often degrade temporal processing along the auditory pathway by evoking neuronal suppression from the auditory nerve on (Harris and Dallos, 1979; Wiggs and Martin, 1998; Joris et al., 2004; Grill-Spector et al., 2006).

To unravel fundamental principles of temporal processing, it is important to stimulate animals with ethologically relevant stimuli in a natural temporal context (Margoliash and Fortune, 1992; Carruthers et al., 2013; Woolley and Portfors, 2013; Theunissen and Elie, 2014; Beetz et al., 2016a). The present study tested, neuronal processing of natural sound sequences, with special focus on the relevance of a natural temporal context, in the inferior colliculus (IC) of the frugivorous bat *Carollia perspicillata*. The IC is considered an important structure for the processing of temporal sound attributes. Collicular neurons are often selective to stimulus parameters such as inter-aural intensity and time differences (Klug et al., 1995), sound duration (Casseday et al., 1994), frequency modulation (Casseday et al., 1997), amplitude modulation (Borina et al., 2008), as well as spectral and temporal sound combinations (combination sensitive neurons; Wenstrup et al., 2012). The increased neuronal selectivity in collicular neurons, compared to the rather unselective neuronal responses of the auditory brainstem, makes the IC an important center for the extraction and integration of sensory stimuli features (Casseday and Covey, 1996; Wenstrup et al., 2012).

We used bats as a model to study the processing of sound sequences, because these animals have to cope with fast time-varying acoustic streams in everyday situations. During echolocation, bats emit high frequency biosonar calls in a repetitive manner. The calls reflect off surrounding objects resulting in echoes. Bats use echoes to detect, localize, and identify objects thus creating an acoustic image of the surrounding (Neuweiler, 1990; Moss and Surlykke, 2010; Kössl et al., 2014; Wohlgemuth et al., 2016). They infer the distance to objects from the echo delay, which represents the time interval between call emission and echo arrival (Hartridge, 1945; Simmons, 1973). Neurons involved in distance processing

respond selectively to call echo pairs, in which the echo follows the call with a certain delay (Grinnell, 1963a; Feng et al., 1978; Suga et al., 1978; Suga and O'Neill, 1979; Hagemann et al., 2010).

Neurophysiological studies in bats revealed that the stimulus repetition rate affects neuronal tuning. Neurons become more selectively tuned to sound duration (Zhou and Jen, 2006), sound frequency (Jen et al., 2001; Smalling et al., 2001), echo delay (O'Neill and Suga, 1982; Wong et al., 1992; Bartenstein et al., 2014), amplitude (Galazyuk et al., 2000), and azimuthal position (Wu and Jen, 1996), when the stimuli are presented at high rates. The aforementioned studies investigated the effect of the stimulus rate on the neuronal tuning by stimulating bats with echolocation sequences composed of constant inter-call interval. However, in real life scenarios, physical parameters like inter-call interval, call duration and the spectral composition of the calls vary during an echolocation sequence (Griffin, 1953; Neuweiler, 1990). Thus, to understand the neuroethological roles of the auditory centers involved in processing echolocation signals, it is necessary to investigate neuronal processing with natural echolocation sequences. So far, processing of natural echolocation sequences has been characterized in the superior colliculus of the insectivorous bat *Eptesicus fuscus* (Wohlgemuth and Moss, 2016) and in the auditory cortex (AC) of *C. perspicillata* (Beetz et al., 2016a, b). Cortical results have shown that the natural temporal context evokes neuronal suppression which results into a high neuronal selectivity to particular call echo pairs (Beetz et al., 2016a) or to object-specific echo information (Beetz et al., 2016b). Yet, it remains largely unknown if the response of subcortical neurons display a sharper echo-delay selectivity when studied with natural echolocation sequences.

Materials and Methods

Animals

Electrophysiological recordings from the inferior colliculus were performed in six adult females of the frugivorous bat *Carollia perspicillata*. Bats were taken from a breeding colony at the Institute for Cell Biology and Neuroscience (Goethe-University Frankfurt, Germany). The animal use in this study complies with all current German laws on animal experimentation and it is in accordance with the Declaration of Helsinki. All experimental protocols were approved by the Regierungspräsidium Darmstadt (experimental permit # F104/57).

Acoustic stimuli

Frequency-level receptive fields were calculated from neuronal responses to pure tones of 10 ms duration (0.5 ms rise-fall time) whose frequency and intensity was varied. Sound frequencies ranged from 5–95 kHz (5 kHz steps) and the sound pressure levels were between 30–90 dB SPL (10 dB steps). Sound levels were adjusted based on the speaker's calibration curve. Each frequency-level combination was randomly presented five times with a 400 ms interstimulus interval.

Natural echolocation sequences were recorded in a pendulum (Henson et al., 1982; Beetz et al., 2016a, b). The bat was placed in a pendulum and swung towards different objects. During the swing, the animal broadcasts echolocation calls. The calls and echoes, arising from call reflections from the surrounding objects, were recorded with an ultrasound sensitive microphone (CM16/CMPA, Avisoft Bioacoustics, Germany). The microphone was attached to the pendulum and positioned medially above the animal's head. The distance between the animals' ears and the microphone membrane was set to 4 cm. The microphone had a sensitivity of 50mV/Pa and an input-referred self-noise level of 18 dB SPL. Sound signals were acquired with an UltraSoundGate 116 Hm mobile recording interface (Avisoft Bioacoustics, Germany) and a sampling rate of 375 kHz (16 bit precision).

For the present study, two representative echolocation sequences recorded in the pendulum were used as acoustic stimuli for electrophysiological recordings. Both sequences were recorded during the forward swing of the pendulum. The pendulum swung at an average speed of 3 m/s (speed calculated as the total x-axis displacement/time). Note that during an approach flight the velocity of *C. perspicillata* ranges between 2-3 m/s (Thies et al., 1998). For the first sequence (simple echolocation sequence in Figure 1A-1E), the bat was swung towards an acrylic glass wall (depth: 0.3 cm; width 50 cm; height: 150 cm). Each echolocation call was reflected once at the acrylic wall. Thus, each call was followed by an echo with a distance-dependent time-delay (defined as echo delay). Echo delays decreased from 22.8 ms to 1.1 ms which correspond to distance changes from 3.9 to 0.17 m (Figure 1C). Echolocation sequence

parameters fell within the natural range of *C. perspicillata* (Table 1 (Thies et al., 1998)). Consistent with findings in freely flying bats (Thies et al., 1998), the call duration decreases as the bat approaches the object in the pendulum paradigm (Figure 1 A).

For recording a multiple-object sequence (Figure 1F-I), three objects were positioned along the swing trajectory. Object A was a dummy rock (depth: 65 cm; width 95 cm; height: 35 cm) made of papier-mâché and it was overflown by the animal at time point t = 450 ms (dashed vertical line in Figure 1 H). Object B, a wooden plate (depth: 0.8 cm; width: 21 cm; height: 21 cm) was positioned 130 cm after object A and 20 cm in front of object C. Object C was the acrylic glass wall from the simple echolocation sequence (see preceding text). The swing of the pendulum stopped directly in front of object B. The objects were positioned so that each echolocation call was followed by at least two echoes. One echolocation call was followed by echoes from all three objects (i.e. call # 8 at t = 0.39 s in Figure 1H). The recorded echolocation sequences were resampled from 375 kHz to 384 kHz. The "Noise Reduction" function (FFT length 256; precision 16) of the software Avisoft SAS Lab Pro (Avisoft Bioacoustics, Germany) filtered background noise in the frequency domain. The spectro-temporal structure of call and echoes were not affected due to the high signal-to-noise ratio of the recording. An elliptic filter (order 8) in the software BatSound (Pettersson Elektronik AB, Sweden) eliminated the remaining sound artifacts from background noise.

For investigating the relevance of the stimulus history, the simple echolocation sequence was cut into segments using a custom-written Matlab script (R2009). Each segment contained a call and an echo. In the rest of the manuscript, we will refer to the segment as "call-echo elements". The call-echo elements were randomly presented with a 400 ms inter-stimulus time interval, from here on called "element situation". The neuronal response to the element situation was compared with the response elicited by the natural echolocation sequence, from here on called "sequence situation".

The multiple-object sequence was transformed into single-object sequences by manually deleting object-specific echoes in the software BatSound (PettersonElektronik AB, Sweden). According to the distances to objects, the echoes from object A and B should be separated by 7.65 ms. Echoes from object B and object C should be separated by 1.2 ms. Calculations are based on the equation:

$$R = D \times \frac{C}{2}$$

R represents the distance, *D* the echo delay and *c* the sound velocity in air at 20° C. In our multi-object sequence, echoes from object A and object B were separated by 6.7 ± 0.9 ms and echoes from object B and object C by 5.3 ± 0.2 ms. The discrepancy of the echo delays between the echoes of object B and object C might be because echoes from object C could derive from the off-axis of the sonar beam. Thus, echoes from object C had to cover longer distances than echoes from object B. Echoes from different objects did not temporally overlap, which allowed us to delete object-specific echoes.

For stimulation, acoustic signals were played using an Exasound E18 sound card (ExaSound Audio Design, Canada) at a sampling rate of 384 kHz. To avoid sound artifacts, such as clicks during stimulation, the acoustic stimuli were multiplied by a fading function resulting in smooth rise-fall times of 0.5 ms. The acoustic stimuli were transferred to an audio amplifier (Rotel power amplifier, RB-850), before they were played through a calibrated speaker (ScanSpeak Revelator R2904/7000, Avisoft Bioacoustics, Germany). The speaker was located 15 cm from the bat's right ear. Speaker calibration was done with a ¼-inch Microphone (Brüel&Kjaer, model 4939, Denmark) connected to a custom-made microphone amplifier.

While recording neuronal signals from the left IC, the sequence situation, the element situation, the multiple-object sequence, and the multiple-object sequences with deleted echoes were presented 15 times with an inter-stimulus interval of 400 ms to an anaesthetized bat. Sound pressure levels of calls and echoes are plotted in Figure 1B and 1F for the simple echolocation sequence and multiple-object sequence, respectively.

Electrophysiological recordings

For anaesthesia, bats were subcutaneously injected with a mixture of ketamine (10 mg × kg−1 Ketavet, Pharmacia GmbH, Germany) and xylazine (38 mg × kg−1 Rompun, Bayer Vital GmbH, Germany). A local anesthetic (Xylocaine 2%, AstraZeneca GmbH, Germany) was applied topically onto the skin of the bat's head. A longitudinal midline cut was made through the skin. Skin and muscles covering the skull were removed. For fixating the bat's head during the recordings, a custom-made metal rod (1-2 cm length, 0.1 cm diameter) was glued with acrylic glue (Heraeus Kulzer GmbH, Germany), super glue (UHU, Germany), and dental cement (Paladur, Heraeus Kulzer GmbH, Germany) at the rostral end of the skull. After two recovery days from surgery, a craniotomy covering an area of 1 mm^2 above the midbrain was done to gain access to the left IC.

Electrophysiological recordings were conducted in a sound-proofed and electrically-shielded chamber. During anesthesia, the temperature of the bat holder was kept constant at 37° with a heating pad positioned below the immobile bat. Neuronal recordings were performed using single glass electrodes (resistance 4–10 MΩ when filled with 3 Mol KCl) which were constructed by pulling borosilicate capillaries (GB120F-10, Science Products, Germany) with a Flaming/Brown horizontal puller (P97, Sutter, USA). Glass electrodes were positioned 2-3 mm lateral from the midline of the scalp. A prominent blood vessel running dorsally over the rostral cerebellum was used as landmark for determining the rostro-caudal position of the IC. The electrode was penetrated orthogonally to the brain surface, through an intact *dura mater*. Recording depths were measured with a Piezo Manipulator (PM 10/1, Science products GmbH, Hofheim, Germany). The brain surface was used as reference point (0 µm) for depth measurement

and the recording depths ranged from 610 µm up to 6210 µm. A silver wire, placed 1-2 cm rostral from the recording electrode and touching the brain surface of non-auditory areas, was used as grounding electrode. Neuronal data acquisition was performed using a wireless multichannel recording system (Multi Channel Systems MCS GmbH, Germany), at a sampling rate of 20 kHz (per channel) and 16 bit precision. One channel of the multichannel recording system was connected to the recording electrode while the remaining channels were short-circuited and connected to ground. One recording session lasted on average 4 hours. Recordings were performed chronically in each animal. After each recording session, the animal had at least one day for recovery. The health status of the animal was documented with health reports, including daily weight measurements.

Analysis of neuronal recordings

Spike events were detected with a multi-unit specific threshold that was based on the spike amplitude. For each multi-unit, spike threshold was kept constant throughout the stimulation protocol, thus ensuring that the same multi-unit activity was recorded for each stimulus. Spike detection was based on spike amplitude relative to recording noise level. The spikes were sorted based on the first three principle components of the spike waveforms and they were clustered automatically using the "KlustaKwik" algorithm (Lewicki, 1998). Only the cluster with the largest number of spikes was used for further analysis. Neuronal responses from the IC were analyzed in 90 spike-sorted single-units.

Initially, the characteristic frequency (CF), which represents the frequency to which the neuron is most sensitive, was calculated for each unit. Neuronal responses to the echolocation sequences were assessed from units with CFs higher than 35 kHz (n = 79). Neuronal data from 149 cortical units from a previous study (Beetz et al., 2016a) were used and compared to the IC data.

A suppression rate calculated with the following equation was calculated for each unit:

$$suppression\,rate = 1 - \frac{\#\,spikes\ (sequence\ situation)}{\#\,spikes\ (element\ situation)}$$

Unless otherwise mentioned, IC data were analyzed with post-stimulus time histograms (PSTHs) with a binsize of 2 ms. The tracking ability of the units was assessed by cross correlating the PSTHs with the down-sampled envelope of the stimulus energy. Note that the PSTH binsize used for the cross-correlation was 1 ms for collicular and cortical units.

The calculation of the signal-to-noise-ratio (Figure 4F) was based on normalized PSTHs. PSTHs were normalized in a unit-specific manner, relative to each unit's maximum spike count per bin when considering both the element and sequence situations. To distinguish between stimulus evoked responses (signal) and background activity (noise), a threshold was set to 50% of the maximum value of the normalized PSTHs. Bins crossing that threshold were defined as the "signal" and compared to the

remaining bins that represent the “noise”. The signal-to-noise ratio of a call-echo element represents the sum of the number of spikes in bins defined as signals divided by the total number of spikes elicited by that call-echo element. Thus, a signal-to-noise ratio of 1 indicates that each spike elicited in response to the call-echo element, was assigned to a “signal” and that the “noise” level was zero. A signal-to-noise ratio was quantified for the responses to each call-echo element. For obtaining a unit specific signal-to-noise ratio, we calculated the median values of the signal-to-noise ratios calculated in response to each call-echo element.

Because of the variable inter-call intervals of the echolocation sequence, conventional PSTHs with constant bin-sizes could not be used to assess the tuning of the collicular units to specific call-echo elements. Therefore, “activity histograms” were calculated by assigning each spike according to its relative time point to the preceding call-echo element (Figure 5A). In other words, activity histograms represent PSTHs with variable binsizes that correspond to the time window of the call-echo elements.

In order to describe delay tuning, two different parameters were calculated. The best delay represents the echo delay (represented by call-echo elements) that elicits the strongest response. The median delay was calculated by measuring the median time point of the evoked spikes. The median time point was then assigned to a call-echo element. The echo delay, encoded by the call-echo element, represents the median delay. In contrast to the best delay calculation, the median delay calculation considers each elicited spike. Data analysis was done in Matlab 2014 (MathWorks, Natick, MA), and statistics in GraphPad Prism 5 (GraphPad Software, USA; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$).

Results

Tonotopy

Extracellular recordings were obtained from 90 auditory sensitive and spike-sorted single-units from the central nucleus of the IC (cIC) of *C. perspicillata*. Recordings were from the cIC because a clear tonotopy was found along the dorso-ventral axis, which is characteristic for the cIC (Figure 2; Grinnell, 1963b; Pinheiro et al., 1991; Schmidt et al., 1991; Sterbing et al., 1994; Jen and Chen, 1998). We determined each unit's characteristic frequency (CF) based on its frequency receptive field (Figure 2B). The CF represents the frequency to which the unit is most sensitive (white stars in Figure 2B). Recording depths were calculated for 85 units and plotted against the CF (Figure 2C). A Spearman cross-correlation analysis and linear regression depict that the CF increased with increasing recording depth ($R = 0.56$, $f(x) = 21.13x+1.06$; $p < 10^{-5}$). Note that the neurons show a multi-peaked receptive field with increasing depth (Figure 2B). Thus, high frequency tuned neurons of the IC receive excitatory input from low (< 35 kHz) and high frequencies (> 35 kHz). Multi-peaked receptive fields have been described for a relatively lower number of neurons in the IC of the mustached bat (Holmstrom et al., 2007), *E. fuscus* (Casseday and Covey, 1992) and *Myotis oxygnathus* (Vasil`ev and Matyushkin, 1967). In the AC of *C. perspicillata*, high frequency tuned neurons are also multi-peaked in *C. perspicillata* (Hagemann et al., 2010; Hagemann et al., 2011).

In comparison to communication signals, echolocation calls have their main energy at high frequencies (Hechavarría et al., 2016a). Therefore, we tested neuronal responses to natural echolocation sequences (see sequences in Figure 1) only in neurons with CFs higher than 35 kHz (79 units positioned to the right of the dashed line in Figure 2D). The remaining eleven units were defined as low frequency tuned neurons and were not taken into consideration for the remaining analysis.

Suppression at IC level is weaker than at the cortical level

We analyzed neuronal responses from 79 collicular units that were recorded while the bats were listening to an echolocation sequence ("sequence situation"; black raster and PSTH (binsize = 2 ms) in Figure 3A). The sequence mimicked a stimulus scenario that the bat could perceive when flying towards an object. To quantify the influence of the temporal context of the sequence on the neuronal response, the bats were also stimulated with the temporally isolated call-echo elements of the sequence. Temporal isolation means that the call-echo elements were randomly presented with a 400 ms inter-element interval (element situation; black and gray raster and gray PSTH in Figure 3A). In comparison to the results from the cortex (Figure 3B), the responses of IC units were less suppressed in the sequence situation (Figure 3A) when compared to the element situation. Neuronal data from 79 collicular and 149 cortical units

(database of cortical units based on Beetz et al., 2016a) demonstrate that the suppression is significantly weaker in the IC than in the cortex (Figure 3C; median: 0.46 and 0.81 for IC and cortex, respectively; Mann Whitney t-test: $p < 10^{-5}$). The suppression was quantified based on a suppression rate that is calculated as the ratio of evoked spikes in the sequence and element situations, and by subtracting that ratio from 1 (for details see Materials and Methods). Note that the IC was not free of suppression which is reflected by suppression rates that differed significantly from 0 (Sign test: $p = 2.6 \times 10^{-22}$).

The time course of the echolocation sequence was more accurately represented by IC than AC neurons (see example raster plots in Figure 3A and 3B). To evaluate the neuronal synchronization to the acoustic events of the echolocation sequence in the sequence situation, the PSTHs (binsize = 1 ms) in response to the element and sequence situation were cross-correlated with the stimulus envelope. High cross-correlation (CC) values indicate a high neuronal synchronization and a high tracking ability of the neurons. In the IC, the CC values did not differ significantly between the element and sequence situation indicating that suppression at the IC did not affect the neuronal synchronization (Figure 3D; median: 0.45 and 0.43 for element and sequence situation, respectively; Wilcoxon signed rank test: $p = 0.86$). In contrast to the IC, cortical neurons less synchronized their discharges to the stimulus envelope. CC values calculated for cortical neurons were significantly lower than the CC values from IC neurons (median: 0.45 and 0.43 for IC and 0.26 and 0.18 for cortex; Kruskal Wallis one-way ANOVA and Dunns-multiple comparison post hoc test: $p < 10^{-5}$). For the cortical neurons, CC values were significantly higher in the element than in the sequence situation (Wilcoxon signed rank test: $p < 10^{-5}$). The degradation of neuronal synchrony was based on the cortical suppression, which is indicated by a negative correlation between suppression rate and CC values (Figure 3E; Spearman: $r = -0.45$; $p < 10^{-5}$; $f(x) = -0.34x + 0.21$). At the level of the IC, no correlation was found between the suppression rate and tracking ability (Pearson: $r = -0.02$; $p = 0.87$).

Collicular suppression increases signal-to-noise ratio

Population activity maps from the IC illustrate the effect of collicular suppression on the neuronal response to the echolocation sequence (Figure 4A, lower panel, and 4B). In the heat-maps, each row represents a normalized PSTH from one collicular unit. Each acoustic event is reliably represented in the response patterns obtained from the element (Figure 4A) and sequence situation (Figure 4B). However, the neuronal response to the sequence was weaker, than the response to the element situation, as indicated by the lighter activity pattern in the heat-maps. The time course of suppression was visualized by subtracting the population activity map of the element situation from that obtained in the sequence situation (i.e. Figure 4B – Figure 4A = Figure 4C). High suppression rates are indicated by bright spots (negative values) in Figure 4C. Suppression occurred mainly during and briefly after neuronal excitation,

as post-excitatory suppression. The latter can be seen when comparing the median PSTHs in response to the sequence (black traces in Figure 4E) and element situations (Figure 4D). The suppression resulted in decreased neuronal activity peaks (compare maximum amplitudes at Figure 4D and 4E). The post-excitatory suppression lowered the median spike rate to zero in some cases (see arrow in Figure 4D and 4E). In response to the element situation, the spiking activity rarely dropped to zero. The post-excitatory suppression increased the signal-to-noise ratio in the sequence compared to the element situation (Figure 4F; median signal-to-noise ratios: 0.33 and 0.17 for sequence and element situation, respectively; Wilcoxon signed rank test: $p < 10^{-5}$).

Collicular suppression sharpens tuning to specific call-echo elements without changing tuning preference

Next, we tested the influence of collicular suppression on the neuronal tuning to call-echo elements of the echolocation sequence. To assess the neuronal tuning to the call-echo elements, each spike recorded in the sequence situation was assigned to the call-echo element that putatively evoked the spike (procedure shown for one example unit in Figure 5A). Each call-echo element was associated to a time window that lasted from call onset to the following call (colored, horizontal bars in Figure 5A represent endpoints of each call-echo element). By using the time windows and the spike time points, each spike was assigned to a call-echo element. For instance, spikes occurring during the first time window (initial green spikes in Figure 5A) were elicited by the first call-echo element. The subsequent blue spikes were putatively evoked by the second call-echo element, and so on. For visualization, alternating green and blue spikes indicate the corresponding call-echo elements to which the spikes were assigned to. Based on the spike assignment, the PSTHs could be transformed into activity histograms (low panels in Figure 5A). The activity histograms of all collicular units were represented as a color-coded population activity map (Figure 5B and 5C). The calculated call-echo element activity maps showed a clear selectivity towards certain call-echo elements. Strong neuronal activity was elicited by the call-echo elements representing intermediate to long echo delays (9-20 ms; Figure 5B and C). Note that the units were sorted in descending order according to the call-echo element eliciting the highest spike rate in response to the sequence situation. Long-delay tuned neurons were positioned at the top and short-delay tuned neurons at the bottom of the activity maps in Figure 5B and 5C. In the sequence situation, collicular suppression lowered the neuronal activity (brighter, more yellowish color in Figure 5C). Suppression also sharpened the neuronal tuning to certain call-echo elements. The sharpening did not significantly change the best delays of the collicular neurons when considering all recorded collicular units (Figure 5D; Wilcoxon signed rank test: $p = 0.13$). In contrast, median delays shifted significantly towards longer delays, indicating that the response to long delays was less suppressed than the response to short delays. In other

words, the strength of suppression increased over time during the stimulus presentation, in some neurons (Figure 5E; Wilcoxon signed rank test: $p < 10^{5}$). Note that the median delay did not change in 39% of the units indicating that collicular suppression did not change delay tuning in all collicular units.

In the IC, information from multiple objects can be processed in parallel

Recent studies showed that cortical neurons process object information from one object (usually the nearest object) when the animals are stimulated with echolocation sequences containing echo information from multiple objects (Beetz et al., 2016b; Greiter and Firzlaff, 2017). Neuronal responses to distant objects are usually suppressed. Since the present study shows that collicular suppression is weaker than cortical suppression, we were interested in determining whether echo information from multiple objects can be processed at the IC level. To address this question, the bats were presented with an echolocation sequence that contained echo information from three objects (multiple-object sequence; Figure 1F-I). The neuronal response was then compared with the response evoked by stimulation with echolocation sequences containing echo information from one object only (single-object sequence). Note that single-object sequences were obtained by manually deleting object-specific echoes from the multiple-object sequence (see Materials & Methods). Thus, single-object sequences contain the same spectro-temporal information as the multiple-object sequence, except for the missing echo information from two out of the three objects. Seventy-seven collicular spike-sorted single-units, with CFs higher than 35 kHz, were tested with the multiple-object sequence and each of the three single-object sequences.

Population activity maps and the median PSTH (binsize = 2 ms) show that each acoustic event evoked a neuronal response when the bats were stimulated with the multiple-object sequence (Figure 6A) and with each single-object sequence (Figure 6B-6D). The temporal bandwidth of PSTHs, obtained in response to the multiple-object sequence, was wider than the one obtained with single-object sequences. The latter becomes obvious when comparing the width of the activity peaks in the median PSTHs with each other (Figure 6A-D, see PSTHs in the bottom subpanels). The width of the activity peaks or the response duration was calculated by autocorrelations of the PSTHs (shown for one example unit in Figure 6E). Autocorrelations were restricted to time lags of ± 20 ms. The larger the area under the autocorrelation curve, the longer is the response duration. The presence of three echoes, instead of one echo, following each call increases the response duration indicating that the collicular unit encoded echo information from more than one object. At the population level, the response duration was also significantly longer when the bats were stimulated with the multi-object sequence than with the single-object sequences (Friedman one way ANOVA and Dunn´s Multiple Comparison Test: $p < 10^{-5}$ in Figure 6F).

A correlation between the PSTHs calculated in response to each single-object and the multi-object sequence allowed for a quantification of the influence from each object on the neuronal response.

Correlation values were highest between PSTHs obtained in response to object B (object B PSTHs) and PSTHs corresponding to the multi-object sequence (multi-object PSTH; median correlation index: 0.57 object A, 0.62 object B, and 0.42 object C; Friedman one way ANOVA and Dunn's Multiple Comparison Test: $p < 10^{-5}$; Figure 7A). Thus, the neuronal response to the multi-object sequence mostly resembles the response to the object B sequence. This result is not surprising because object B contributes more echoes to the multiple-object sequence (17 echoes) than object A (8 echoes) or object C (7 echoes). Thus, the highest stimulus similarity was already biased towards object B in the multiple-object sequence. Note that stimulus similarity does not exclusively account for the differences in the calculated correlation values (Figure 7A). Object A and object C provide about the same number of acoustic events to the multiple-object sequence. If the correlation values were simply reflecting the amount of acoustic events, then object A and object C should comparably influence the response to the multiple-object sequence. Yet, object A PSTHs were more similar to the multiple-object PSTH than object C PSTHs (Figure 7A). Note that the intensity of the echoes from object A were usually higher than from object C. Therefore, intensity driven influences cannot be excluded with the stimulus setting used in the present study. Next, we determined which of the three objects influenced most the multi-object-evoked PSTH. The object resulting in the highest correlation value was determined for each unit. As expected from the previous results, object B had the highest impact in the response to the multiple-object sequence in most neurons (73%; Figure 7B). However, the multi-object PSTH was most similar to the object A and object C PSTHs in 27% and 1% of the units, respectively.

In the next step, we tested whether the collicular neurons shifted their tuning preferences to the objects, during the presentation of the multi-object sequence. For quantification, the PSTHs (binsize 2 ms) were cut into 21 PSTH segments. Each PSTH segment contained 40 ms of neuronal activity (for details see Materials and Methods). Note that the 40 ms time window correspond to the time window used for the autocorrelation analysis in Figure 6. Correlation values between the segmented multiple-object PSTH and each segmented single-object PSTH were plotted as boxplots as a function of temporal position of each segment in the sequence (Figure 7C). Before passing object A at the 450 ms mark (black vertical dashed line in Figure 7C), object A most strongly determined the neuronal response to the multiple-object sequence. This is indicated by significantly higher correlation values in five out of eleven PSTH segments (depicted by an "A" above the boxplots in Figure 7C). After passing object A, only echoes from object B and object C were present. In that situation, object B had the strongest influence on the multi-object PSTH, as indicated by higher correlation values in seven out of ten PSTH segments (depicted by a "B" above the boxplots in Figure 7C). Correlation values between the segmented object C PSTH and the segmented multiple-object PSTH were never significantly higher than the ones between the segmented multiple-object PSTH and the segmented object A and object B PSTHs. In summary, the initial 450 ms of the response pattern to the multi-object sequence is predominantly determined by echo information

coming from object A. After passing object A, echoes from object B have the strongest influence on the response pattern to the multi-object sequence. Although collicular neurons responded to each echo of the multi-object sequence, collicular suppression ensures that echoes from the nearest object are mostly determining the response pattern to the multi-object sequence.

Discussion

In the auditory system, neuronal spikes are usually synchronized to the stimulus envelope. Along the ascending auditory pathway, the cut-off frequency that can elicit such synchronization decreases (for review see: (Joris et al., 2004; Wang et al., 2008; Simmons and Simmons, 2011). When stimulating animals with acoustic rates higher than 40 Hz, cortical neurons are sometimes completely suppressed. Thus, neuronal suppression degrades temporal processing of repetitive stimuli. With this in mind, one could ask how neurons of animals that behaviorally rely on high repetition rates of sensory information cope with such suppression effect? The present study quantified the influence of the temporal context (present in natural echolocation sequences) on the response of IC neurons of bats. The echolocation sequences used by us mimic a stimulus situation encountered by the bat when approaching one or several target objects.

Neuronal suppression does not necessarily degrade temporal processing

The cut-off frequencies of mammalian collicular neurons are heterospecific and range between 10-1000 Hz (~ 100-150 Hz gerbils (Krishna and Semple, 2000), and guinea pigs (Rees and Palmer, 1989), squirrel monkeys (Müller-Preuss et al., 1994), ~ 200 Hz in rats (Rees and Moller, 1983), ~ 1000 Hz in cats (Langner and Schreiner, 1988)). Cut-off frequencies of bat collicular neurons range between 94-400 Hz (~ 100 Hz in *Rinolophus rouxi* (Reimer, 1987) and ~94-400 Hz in *M. lucifigus* (Condon et al., 1994)). Overall, the results from previous studies would suggest that bat IC neurons should be able to synchronize their spiking to echolocation sequences in which the repetition rate never reaches 100Hz, as it is the case in *C. perspicillata*. Our results confirm this prediction. We show that collicular neurons of *C. perspicillata* synchronize their discharges to the stimulus envelope of each acoustic signal in the echolocation sequences (Figure 3). Note that this result is contrasted by findings from studies in the Mexican free-tailed bat, which show that some collicular neurons respond selectively to particular syllables of a communication sequence (Andoni and Pollak, 2011). This discrepancy could arise from heterospecific effects or from using different types of acoustic stimuli, i.e. echolocation compared to communication signals.

Despite the neuronal synchronization in the IC of *C. perspicillata*, the collicular neurons were suppressed in the sequence situation. However, instead of degrading temporal processing, collicular suppression improved temporal processing by increasing the signal-to-noise ratio (Figure 4) and the neuronal selectivity to particular call-echo elements (Figure 5). In agreement with our results, numerous studies have reported that subcortical neurons sharpen their neuronal tuning with increasing repetition rates (IC: *M. lucifugus* (Friend et al., 1966; Galazyuk et al., 2000); *E. fuscus* (Pinheiro et al., 1991; Chen and Jen, 1994; Moriyama et al., 1994; Wu and Jen, 1996; Jen and Chen, 1998; Jen and Zhou, 1999; Jen et

al., 2001; Zhou and Jen, 2001; Zhou and Jen, 2002; Sanderson and Simmons, 2005; Wu and Jen, 2006; Zhou and Jen, 2006; Jen and Wu, 2008); superior colliculus: *E. fuscus* (Valentine and Moss, 1997; Wohlgemuth and Moss, 2016)). Some studies even described repetition rate selective neurons in the IC of insectivorous bats, like *E. fuscus*, (Pinheiro et al., 1991; Sanderson and Simmons, 2005) and *M. lucifugus* (Condon et al., 1994). Repetition rate or inter-syllable interval selective neurons have been described in different animals, including crickets (Zorovic and Hedwig, 2011), fishes (Crawford, 1997), frogs (Rose, 2014; Rose et al., 2015), and birds (Araki et al., 2016). Crickets (Hedwig, 2006), frogs (Feng et al., 1990), and presumably birds (Araki et al., 2016) identify conspecifics by determining species-specific repetition rate or inter-syllable interval of the acoustic signals. In bats, an improvement in temporal tracking with signal repetition rate could be of advantage for information extraction during echolocation. Some bat species increase their call rate from 10 to 200 Hz during an approach flight that ends with an insect capture (Simmons et al., 1979). Thus, specific neuronal populations are excited at different hunting stages (Jen and Schlegel, 1982; Condon et al., 1994). In contrast to insectivorous bats, frugivorous bats such as *C. perspicillata* change less dramatically their call rates during echolocation (*C. perspicillata:* (Thies et al., 1998), *P. discolor:* (Linnenschmidt and Wiegrebe, 2016)). During approach flights, *C. perspicillata* increases the call rate from 12 ± 19 Hz to 24 ± 39 Hz (Thies et al., 1998). Even though frugivorous bats change their call rates less prominently than insectivorous bats, the former could still profit from and enhanced temporal representation of acoustic streams at the level of the IC.

Temporal selectivity increases from the IC to the AC

By using the same stimulus settings in the IC and AC (Beetz et al., 2016a), it is possible to compare the influence of a natural temporal context in both brain areas for the first time. Cortical neurons of *C. perspicillata* have a cut-off frequency of 20 Hz (Martin et al., 2017). To modulation (Martin et al., 2017) or repetition rates (Beetz et al., 2016a; Hechavarría et al., 2016b) higher than 20 Hz, cortical neurons become suppressed and they respond exclusively to certain call-echo elements (Beetz et al., 2016a). The results from this study show that collicular suppression is weaker than the cortical one (Figure 3C) and that main suppressive effects seen in the AC may arise at the thalamus or cortex, as proposed by findings from rodents (Wehr and Zador, 2005; Bayazitov et al., 2013). Note that although suppression is weaker in the IC, the time course of suppression is comparable between the cortex and midbrain (for IC data see gray trace in Figure 4E). Main suppressive effects occurred during or directly after strong excitations. Post-excitatory suppression is common in the mammalian cortex (Suga et al., 1983; Joris et al., 2004; Beetz et al., 2016a) and has also been described in the IC of different bat species, including *E. fuscus* (Covey et al., 1996), *Tadarida brasiliensis mexicana* (Bauer et al., 2000), and *M. lucifugus* (Voytenko and Galazyuk, 2007).

An increase of neuronal selectivity for temporal parameters or specific vocalizations along the processing pathway has been demonstrated in different animals, including crickets (Schildberger, 1984; Zorovic and Hedwig, 2011; Kostarakos and Hedwig, 2012; Schöneich et al., 2015), fishes (Partridge et al., 1981), frogs (Rose and Capranica, 1983; Feng et al., 1990), birds (Margoliash, 1986; Doupe and Konishi, 1991; Lewicki and Arthur, 1996; Fujimoto et al., 2011; Theunissen and Elie, 2014), and mammals (Wang et al., 1995; Beetz et al., 2016a). Findings from the auditory cortex of rats demonstrated that hearing selectivity is refined by selective inhibition during development (Chang et al., 2005). The present results from *C. perspicillata* indicate that hearing selectivity along the ascending auditory pathway could be a least partially shaped by time-dependent neuronal suppression. We base this claim on the fact that neuronal selectivity to particular call-echo elements was higher in the sequence than in the element situation (present study and Beetz et al., 2016a). The latter holds true for both the IC and AC, but suppression does have the strongest effects at the level of the AC.

Relevance of the stimulus history for neuronal processing

The sensory world continuously changes and preceding stimuli determine how subsequent stimuli are processed (Brosch et al., 1999; Näätänen et al., 2001; Bartlett and Wang, 2005; Dehaene et al., 2015). In birds, the significance of stimulus history, represented by the temporal context and stimulus order, has been widely demonstrated in neurophysiological experiments. Neurons of the auditory forebrain, respond most strongly to syllables of the bird's own song when presented in the natural temporal context (Margoliash, 1983, 1986; Margoliash and Fortune, 1992). The neurons respond less selectively when presenting the syllables temporally isolated (Margoliash, 1983; Margoliash and Fortune, 1992), as in the element situation of the present study, or when presenting the bird's song in a reversed manner (Margoliash, 1986; Margoliash and Fortune, 1992; Volman, 1996). In mice, neurophysiological experiments demonstrated that cortical neurons respond most strongly to ultrasonic vocalizations when the mice were stimulated with a natural temporal context (Carruthers et al., 2013). In bats, the neuronal selectivity to echolocation (present study and Beetz et al., 2016a) and communication signals (Esser et al., 1997; Hechavarría et al., 2016b) is also highest when stimulating with the natural temporal context.

Although, presenting the call-echo elements in a chronological order, in the present study, it is possible that neuronal tuning could additionally depend on the order of call-echo elements in the sequence. Order selective neurons have been characterized in rats (Kilgard and Merzenich, 2002; Nakahara et al., 2004), birds (Lewicki and Arthur, 1996; Doupe, 1997), and monkeys (Ninokura et al., 2004; Yin et al., 2008; Sadagopan and Wang, 2009; Berdyyeva and Olson, 2010; Crowe et al., 2014). The present study demonstrates the importance of not only using natural stimuli but also presenting the stimuli in the natural temporal context (Theunissen and Elie, 2014).

Target distance processing in the IC of *C. perspicillata*

To our knowledge, this is the first study characterizing the properties of delay tuning in the IC of *C. perspicillata*. In *P. parnellii*, delay tuned neurons of the IC usually respond only to a combination of call and echo but not, or only sparsely, to call or echo alone (Yan and Suga, 1996; Macías et al., 2016). In contrast, collicular neurons of *E. fuscus*, another insectivorous species respond in 59% of the recordings to call and echo (Sanderson and Simmons, 2005). In *C. perspicillata*, the collicular units usually respond to call and echo which is indicated by two activity peaks per call-echo element in the median PSTHs (Figure 4D and 4E). Neuronal tuning to certain call-echo elements was only visible when integrating the number of spikes elicited by each call-echo element.

In the midbrain of *E. fuscus* best delays shorter than 8 ms are rare (IC: Dear and Suga, 1995; superior colliculus: Valentine and Moss, 1997). We also did not find best delays shorter than 8 ms in the present study (mean "best delay" = 14.4 ± 6 ms; mean "median delay" = 10.5 ± 2.1 ms; Figure 5C). However, delay tuning to short delays has been well characterized in the mustached bat's IC (Mittmann and Wenstrup, 1995; Portfors and Wenstrup, 1999; Wenstrup and Portfors, 2011). These different findings may be due to interspecific differences. Although, it is tempting to compare the present results with studies on delay tuning from other bat species, it is noteworthy that we characterized delay tuning based on natural acoustic stimuli while previous studies used mostly artificial signals that mimicked the bat's call-echo pairs presented in isolation (Kössl et al., 2014). Contrary to the IC, in the cortex od *C. perspicillata*, a number of neurons do respond to echo delays shorter than 8 ms. The latter occurs regardless of whether natural sequences or artificial pulse-echo elements are used for calculating the tuning (Hagemann et al., 2011; Beetz et al., 2016a). Future studies could assess whether the short-delay tuning found in *C. perspicillata*'s AC is created along the colliculo-cortical axes, or whether it exists in regions of the IC that were not targeted in the present study. Previous studies have shown that in the cortex, GABA-mediated inhibition can change the best delay of the neurons (Xiao and Suga, 2004; Hechavarria and Kössl, 2014). The latter could be a mechanism for modifying the delay tuning that is already established at the level of the IC.

Collicular responses to multiple echoes

Only a few studies have characterized how bat auditory neurons extract information from multi-echo biosonar sequences (Edamatsu and Suga, 1993; Sanderson and Simmons, 2002; Beetz et al., 2016b; Greiter and Firzlaff, 2017). This stimulus setting mimics the presence of multiple objects or multi reflective surfaces. One study in the IC of *E. fuscus* tested the neuronal processing of "spectral notches". Such spectral notches derive from two temporally overlapping echoes (Sanderson and Simmons, 2000).

Note that in the preset study spectral notches do not occur since temporally non-overlapping echoes were used to create our stimulation sequence. Non-overlapping echoes were created by the presence of up to three objects located at different distances from each other.

Previous data from the AC of *C. perspicillata* showed that the neurons preferentially process echo information from the nearest object (Beetz et al., 2016b). The data from anaesthetized bats imply that neurons focus by default on the nearest object and that this processing strategy works without the attention of the animal. However, recent behavioral experiments from *Pipistrellus kuhlii* (Amichai and Yovel, 2017) and from *P. abramus* (Fujioka et al., 2016) demonstrate that bats can attend to distant objects, even in the presence of immediate objects. Such behavioral results clearly go beyond the neurophysiological results from the anaesthetized bat that was stimulated with a natural echolocation sequence (Beetz et al., 2016b). Although the animal's attention could affect neuronal processing, the behavior observed in *P. kuhli* and *P. abramus* could also be accomplished with neuronal information from the IC. Collicular neurons keep track of echoes from multiple objects (Figure 7). In the AC of *P. discolor*, most neurons also respond to the nearest object, but a small population of neurons preferably responded to a more distant object (Greiter and Firzlaff, 2017). These results resemble more the IC results of the present study than the AC results from *C. perspicillata* (Beetz et al., 2016b). The different results from *C. perspicillata* and *P. discolor* could be based on heterospecific differences but differences in the used acoustic stimuli cannot be discarded. Natural hearing is an active process that requires the animal's attention (Theunissen and Elie, 2014) and neurons involved in auditory feedback in self-vocalizations have been characterized (Schuller, 1979; Radtke-Schuller and Schuller, 1995). Therefore, behavioral results should be cautiously correlated with neurophysiological results from anaesthetized and passively listening bats.

Neuroethological roles of the IC for echolocation in bats

Bilateral ablation experiments of the main nucleus of the IC showed that the IC is required for echolocation (Suga, 1969b). In comparison, cortical ablation and focal inactivation of the AC less severely affected the bats' echolocation behavior (Suga, 1969a; Riquimaroux et al., 1991). The IC projects to and receives input from different motor centers (Schweizer, 1981; Covey et al., 1987; Olazabal and Moore, 1989; Moriizumi and Hattori, 1991; Schuller et al., 1991; Wenstrup et al., 1994). Therefore, the IC is discussed to be important for control of fast motor commands and reflexive behaviors during echolocation (Casseday and Covey, 1996). The results presented in this study corroborate this idea because the temporal structure of the echolocation sequence is highly preserved at the collicular level. During echolocation, bats need to integrate and possibly predict echo information. Collicular neurons convey time stamps of the echolocation signals. The latter could be important for predictive coding in high brain areas (Wacongne et al., 2012). Despite the high tracking ability of collicular neurons (Figure 3 and 4), IC units

were selective to specific call-echo elements (Figure 5) and to object-specific echoes (Figure 7). Based on our findings, one could speculate that IC responses allow parallel processing of multiple auditory streams, with a certain selectivity to specific echo delays.

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Table and Figures:

Table 1: Temporal call parameters of the echolocation sequences, used in the present study, are compared with call parameters measured in the field (from Thies et al. 1998).

	call duration [ms]	call interval [ms]	duty cycle [%]
simple echolocation sequence	0.79 ± 0.15	40 ± 14.85	2 ± 0.56
multi-object sequence	1.29 ± 0.25	49.33 ± 20.9	3.06 ± 1.14
freely flying (from Thies et al. 1998)	0.8 ± 0.2	42.2 ± 25.8	2.4 ± 1.1

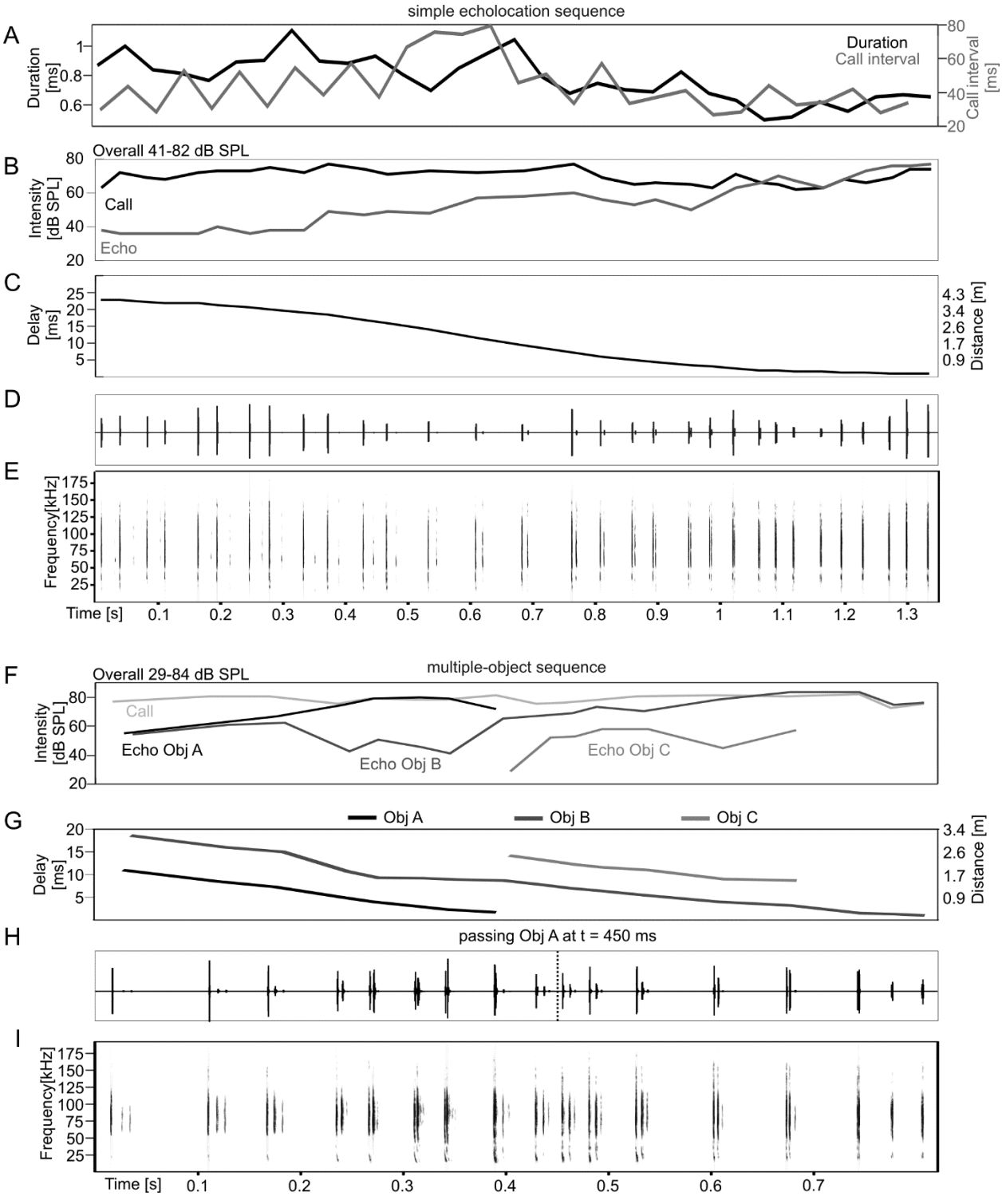
simple echolocation sequence
A
Duration [ms]
Duration
Call interval
Call interval [ms]
B
Overall 41-82 dB SPL
Intensity [dB SPL]
Call
Echo
C
Delay [ms]
Distance [m]
D
E
Frequency[kHz]
Time [s]
F
Overall 29-84 dB SPL
multiple-object sequence
Intensity [dB SPL]
Call
Echo Obj A
Echo Obj B
Echo Obj C
G
Obj A
Obj B
Obj C
Delay [ms]
Distance [m]
H
passing Obj A at t = 450 ms
I
Frequency[kHz]
Time [s]

Figure 1: Natural echolocation sequences used as acoustic stimuli

Two representative echolocation sequences recorded during a forward swing of the pendulum. (A-E) Energetic, spectral and temporal parameters characterizing the simple echolocation sequence. The sequence contains echo information from one object (acrylic glass wall positioned at the end of the swing). (A) Call duration (black trace) and call interval (gray trace) over the time course. Call durations and intervals decrease towards the end of the swing. (B) Call intensity is independent from object distance and varies between 67-82 dB SPL. Echo intensity increases during the approach from 41 to 82 dB SPL. (C) Echo delay decrease over time. (D) Oscillogram and (E) spectrogram of the simple echolocation sequence. (F-H) Same plots as in (B-E) but with physical parameters from the multiple-object sequence. During the swing the bat faced three objects. Thus, each call was followed by at least two echoes coming from different objects. Object A is overflown by the animal between 400 and 450 ms. Therefore, echolocation signals after 450 ms do not contain echo information from object A.

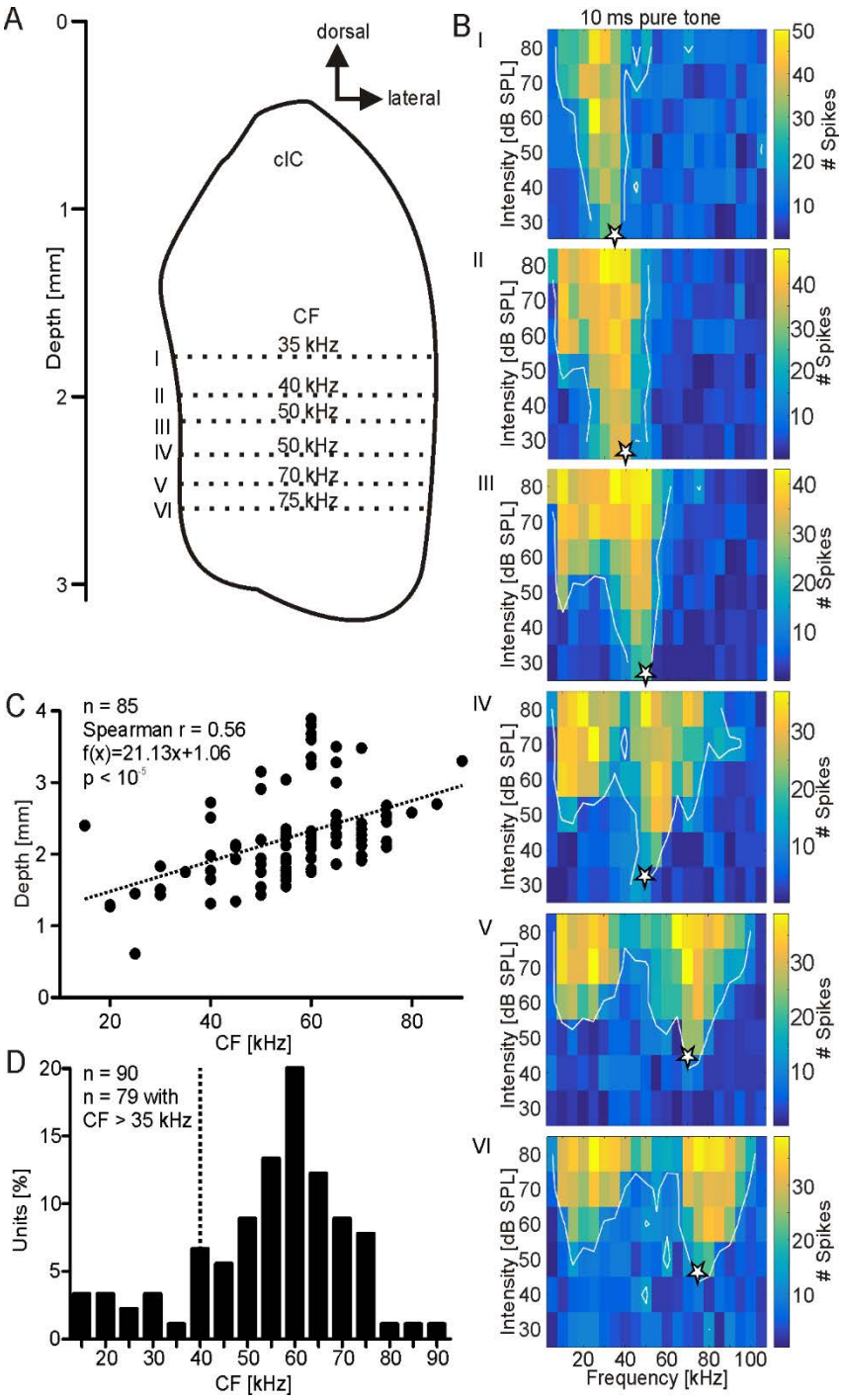

Figure 2 Tonotopy of the central nucleus of the inferior colliculus (cIC) in *C. perspicillata*

(A) Schematic frontal view on the cIC. Characteristic frequencies (CFs) increased with recording depth. (B) Representative frequency receptive fields from six units recorded from different depths of one penetration track. Depths are indicated by roman numbers (I-VI) in (A). The CFs of the units are indicated by white stars in the receptive fields and increase with the recording depths. High frequency tuned neurons typically had multipeaked frequency receptive fields. (C) Scatter plot shows the increase of the CF along the recoding depth for 85 collicular units. (D) Histrogram represents the distribution of CFs from 90 collicular units recorded in the present study. Units with CFs higher than 35 kHz (dashed vertical line) were classified as high frequency tuned units and were tested further with the echolocation sequences from Figure 1.

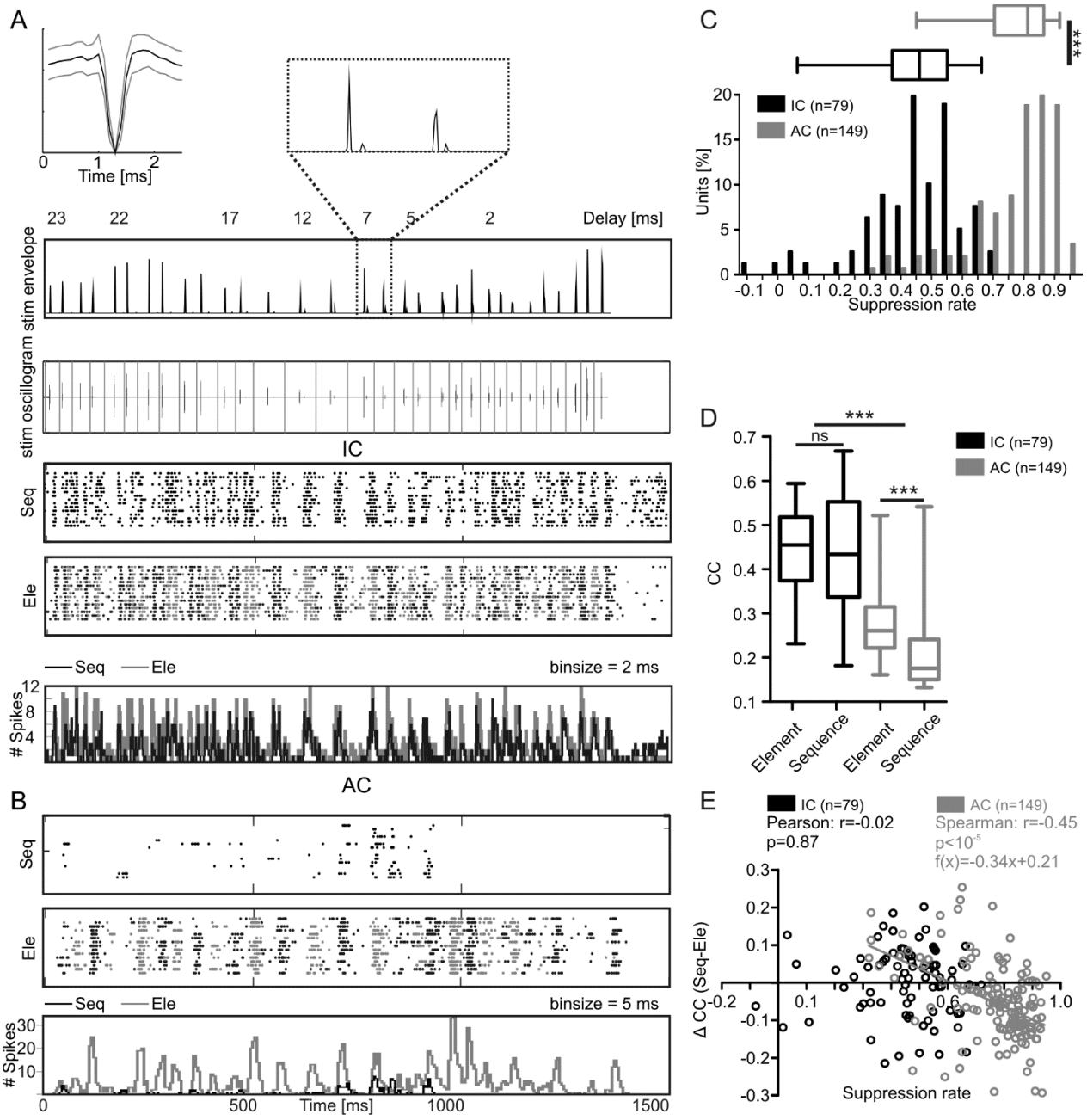

Figure 3: Collicular neurons synchronize more their discharges to the stimulus envelope than cortical neurons.

(A) Neuronal response from a representative unit of the inferior colliculus (IC). Median (black trace), 25th, and 75th quantile (gray traces) spike waveform is shown in the left upper corner. Stimulus envelope and oscillogram of the stimulus are shown below. Two call-echo elements of the stimulus envelope are magnified on top. Neuronal responses are shown as raster plots, where one dot indicates an action potential, and as post stimulus time histograms (PSTH). In the sequence situation (Seq) the animals were stimulated with the natural echolocation sequence. In the element situation (Ele) single call-echo elements of the sequence were randomly presented with a 400 ms interstimulus time interval. The time borders of the call-echo elements are indicated by grey vertical lines in the oscillogram. For visualization, the raster plot of the element situation was created by concatenating the neuronal responses to the call-echo elements. Alternating gray scales visualize which action potentials were evoked by which call-echo

element. (B) Neuronal response from a unit of the auditory cortex (AC) to the sequence and element situation. Raster plots and PSTHs are organized as in (A), except that the binsize of the PSTH was adjusted to 5 ms. (C) Boxplot and histogram of the suppression rates in the IC and AC. In the sequence situation IC units were less suppressed than AC units (Mann Whitney t-test: $p < 10^{-5}$). (D) Boxplots showing the cross-correlation (CC) values calculated between PSTHs, with a binsize of 1 ms, and the stimulus envelope. In the IC (black boxplots) the CC values did not differ between the element and sequence situation ($p > 0.05$), indicating that subcortical suppression prevails the neuronal synchronization to the stimulus envelope. CC values from the AC (grey boxplots) were significantly smaller than in the IC ($p < 10^{-5}$) and decreased further from the element to the sequence situation ($p < 10^{-5}$). Wilcoxon signed rank test for testing between stimulus conditions and Kruskal Wallis one-way ANOVA and Dunns-multiple comparison post hoc test for comparing between IC and AC. (E) Scatter plot shows that in AC (grey circles) the suppression rate was correlated with the decrease of neuronal synchronization from the element to the sequence situation (Spearman: $r = -0.45$; $p < 10^{-5}$, $f(x) = -0.34x + 0.21$). No correlation between the suppression rate and changes in neuronal synchronization was found in the IC (black circles; Pearson: $r = -0.02$; $p = 0.87$).

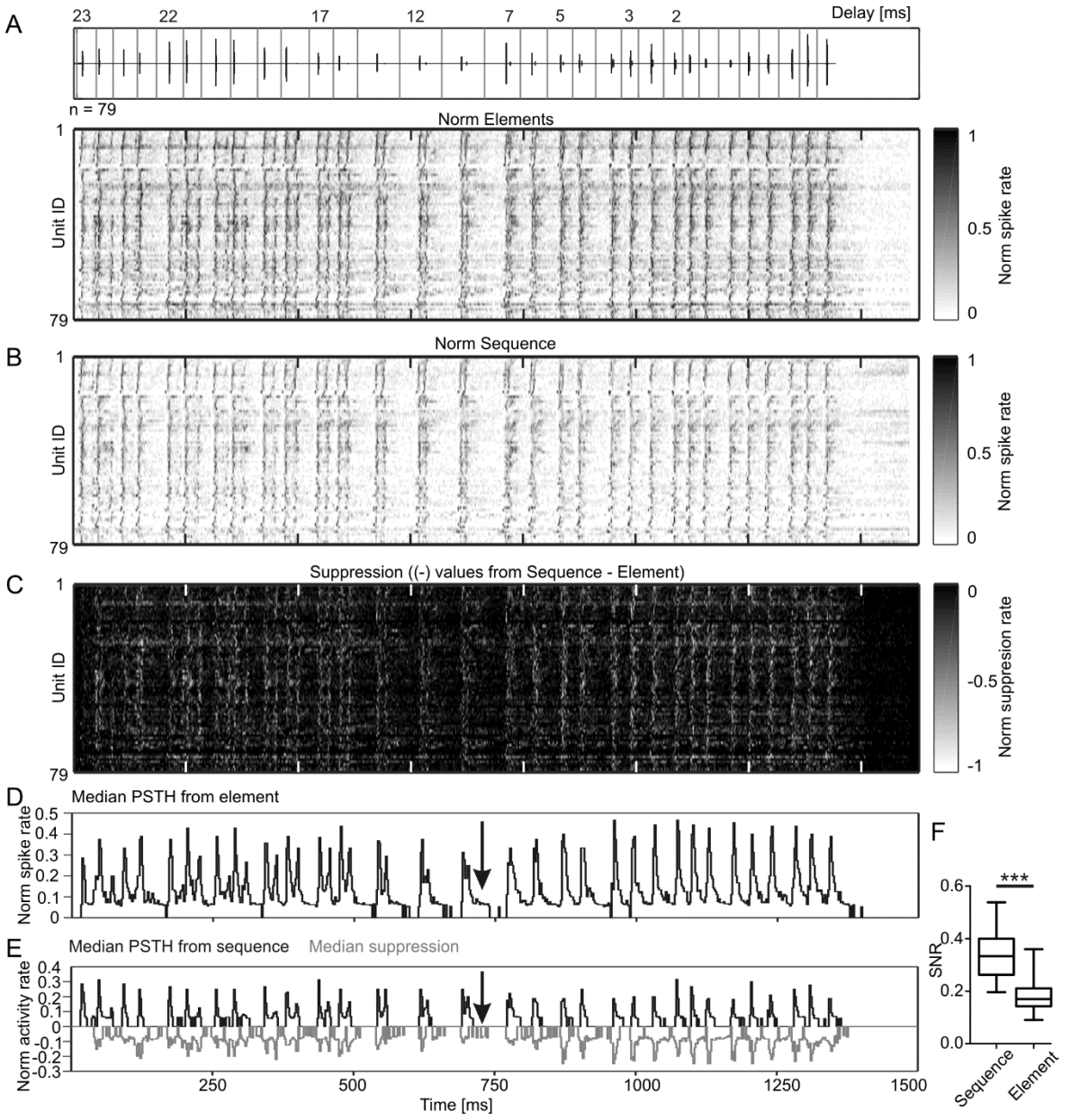

Figure 4 Collicular suppression increases signal-to-noise ratio

(A) Top: Oscillogram of the stimulus. Vertical gray lines define the time borders of the call-echo elements. Bottom: Population activity map in response to the element situation. Normalized PSTHs were transformed into grayscale coded activity maps. Neuronal activity from each unit is represented in a single row. (B) Population activity map in response to the sequence situation. (C) Population suppression map calculated by subtracting population activity map in response to the element situation (A) from the map calculated in response to the sequence situation (B). Respectively, bright and dark bins represent high and weak suppression rates. (D, E) Median PSTHs calculated form the response to the element (D) and sequence (black PSTH in E) situation. The time course of the median suppression is plotted in grey. Note that strong suppression occurs during and directly after high activity rates. The latter suppression reduces the post-activity to zero (black arrows). (F) Boxplots showing the increase in the signal-to-noise ratio in the sequence situation compared to the element situation. Wilcoxon signed rank test: $p < 10^{-5}$. norm = normalized; SNR = signal-to-noise ratio.

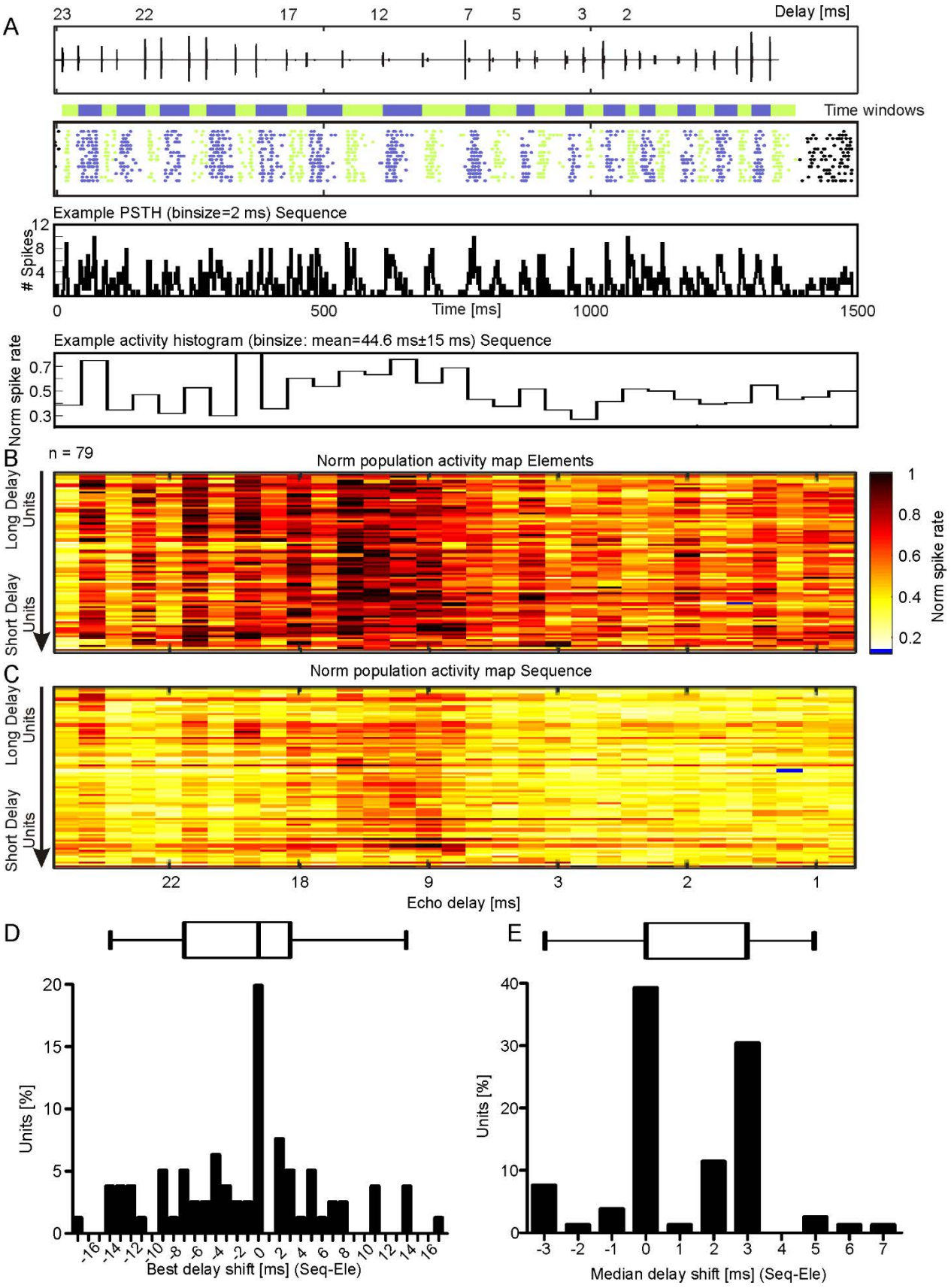
A
23
22
17
12
7
5
3
2
Delay [ms]
Time windows
Example PSTH (binsize=2 ms) Sequence
Spikes
12
8
4
0
500
Time [ms]
1000
1500
Example activity histogram (binsize: mean=44.6 ms±15 ms) Sequence
Norm spike rate
0.7
0.5
0.3
B
n = 79
Norm population activity map Elements
Long Delay Units
Short Delay Units
1
0.8
0.6
0.4
0.2
Norm spike rate
C
Norm population activity map Sequence
Long Delay Units
Short Delay Units
22
18
9
3
2
1
Echo delay [ms]
D
Units [%]
20
15
10
5
0
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
Best delay shift [ms] (Seq-Ele)
E
Units [%]
40
30
20
10
0
-3
-2
-1
0
1
2
3
4
5
6
7
Median delay shift [ms] (Seq-Ele)

Figure 5 Collicular suppression sharpens neuronal tuning to call-echo elements

(A) Oscillogram of the echolocation sequence. Neuronal response of the example unit from Figure 3A is shown as raster plot and PSTH (binsize = 2 ms). To investigate neuronal tuning to certain call-echo elements, each spike was assigned according to its time point to a call-echo element. Time windows used for spike assignments to corresponding call-echo elements are indicated by alternatingly colored horizontal bars. The spikes assigned to a time window and thus to a call-echo element are correspondingly color coded. The activity rate was plotted against the call-echo elements which can be characterized based on their echo delay (x-axis). Note that, the depicted unit responded more strongly to long than to short delays, having its maximum response at element #8 (best delay of 20 ms). (B, C) Normalized population activity maps in response to the element (B) and sequence (C) situation. Units were ordered along the y-axis according to their best delay calculated from the response to the sequence. (D, E) Boxplots and histograms represent the best delay (D) and median delay shifts (E), calculated by subtracting the best or median delay in response to the element situation from the best or median delay in the sequence situation, respectively.

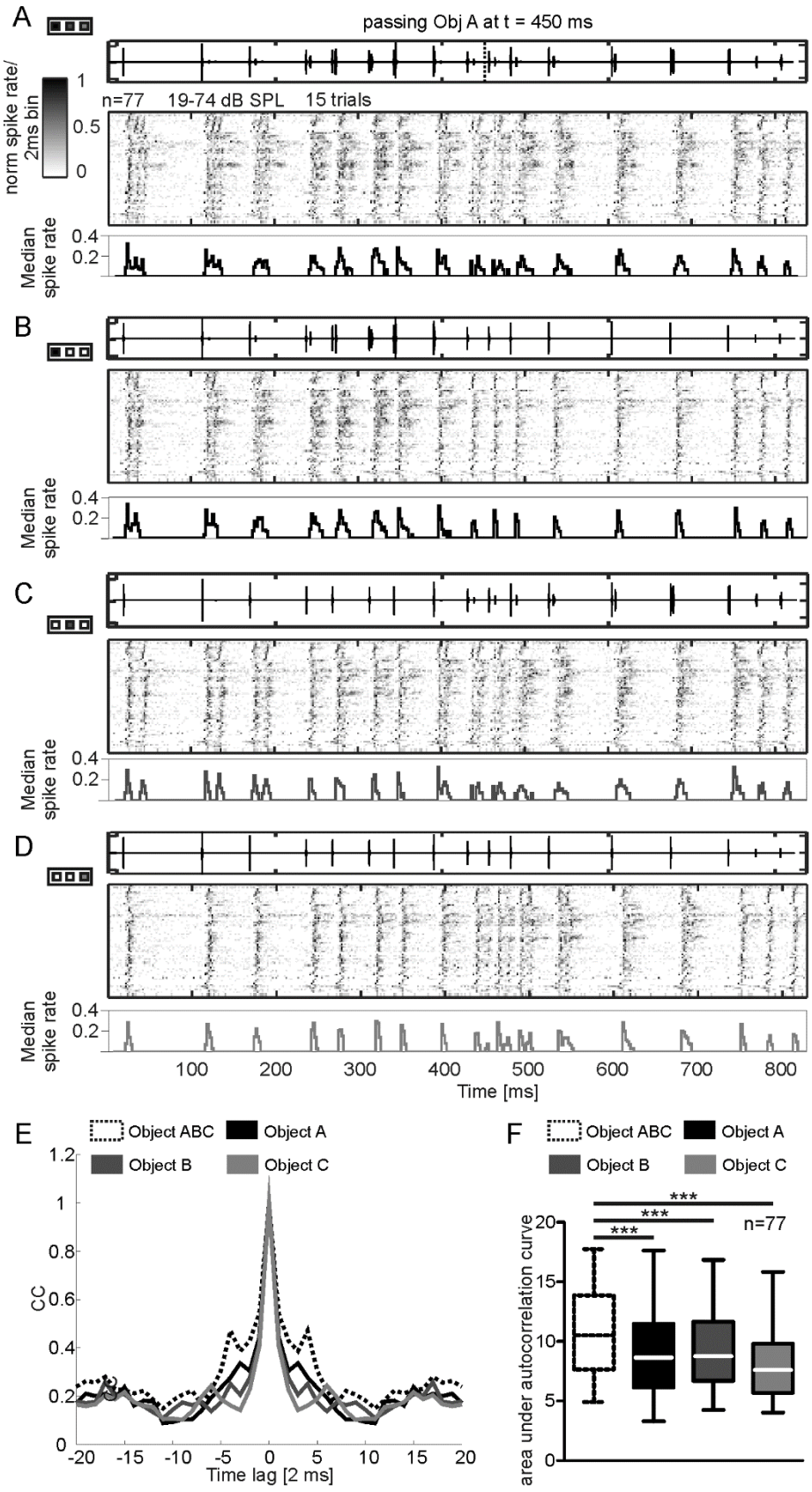
A
passing Obj A at t = 450 ms
norm spike rate/ 2ms bin
1
0.5
0
n=77 19-74 dB SPL 15 trials
Median spike rate
0.4
0.2
B
C
D
100
200
300
400
500
600
700
800
Time [ms]
E
Object ABC
Object A
Object B
Object C
CC
1.2
1
0.8
0.6
0.4
0.2
0
-20
-15
-10
-5
0
5
10
15
20
Time lag [2 ms]
F
***
n=77
area under autocorrelation curve
20
15
10
5
0

Figure 6 Neuronal response to the multi-object sequence

(A-D) Stimulus oscillograms, population activity maps (binsize = 2 ms), and median PSTHs from 77 collicular units in response to the multi-object (A), object A (B), object B (C), and object C (D) sequence. Legends on the left side from each oscillogram define the position of the object along the swing trajectory. Note that each acoustic event, including calls and echoes, is represented in the response pattern. (E) Auto-correlation functions of the PSTHs from an example unit indicated by arrows in (A-D). The auto-correlation function of the PSTH in response to the multi-object sequence is wider than the one of the PSTHs in response to the single-object sequences. (E) Statistical comparison of the area under the auto-correlation curves of 77 collicular units indicate that the response to the multi-object sequence was broader than the response to the single-object sequences (Friedman one-way ANOVA and Dunn´s Multiple Comparison Test: $p < 10^{-5}$). CC = cross-correlation values of the auto-correlation.

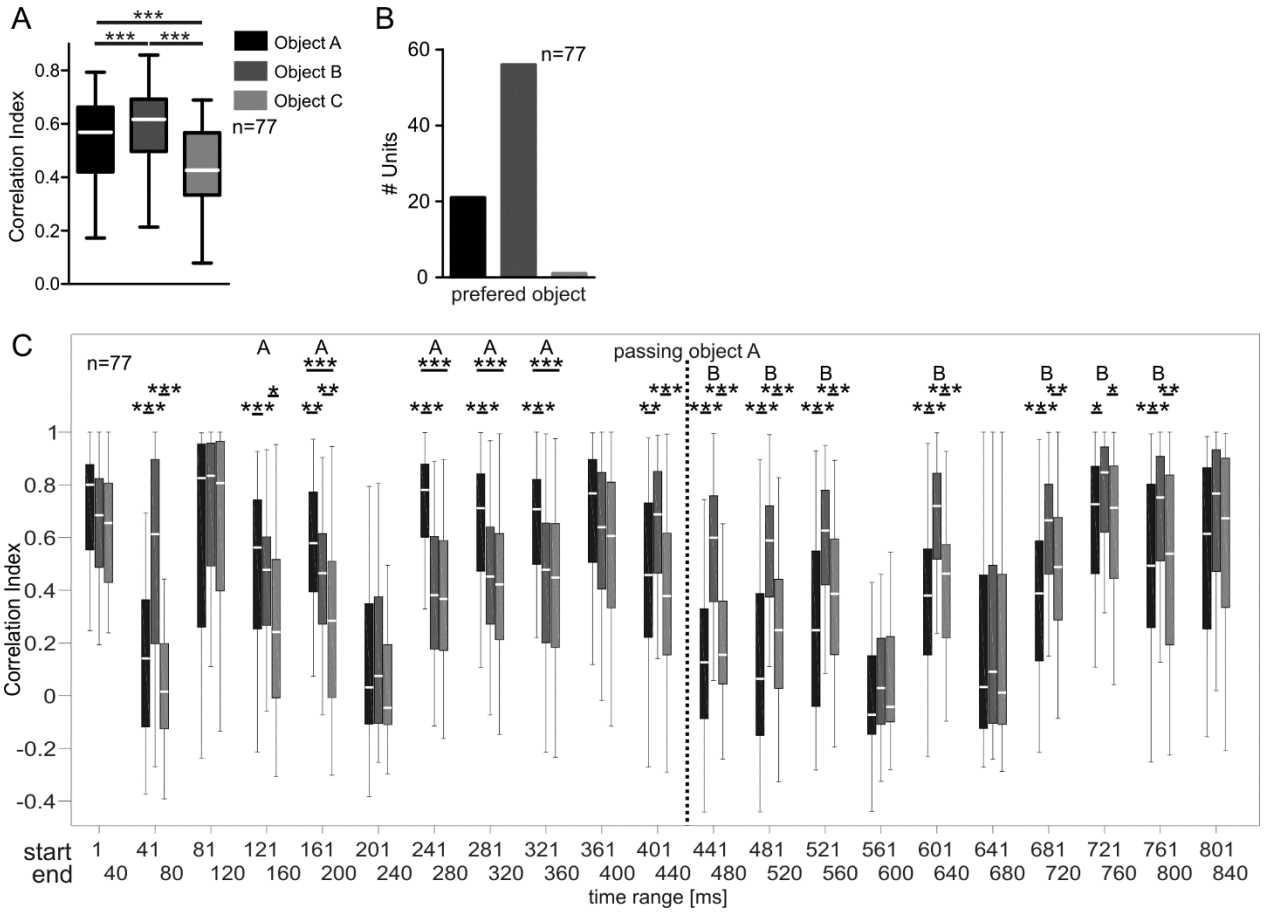

Figure 7 Influence of each object on the neuronal response to the multi-object sequence

(A) Correlation values between each single-object PSTH (object A, object B and object C PSTH) and the multi-object PSTH are plotted as boxplots. Object B PSTH had the highest similarity to the multi-object PSTH (Friedman one-way ANOVA and Dunn´s Multiple Comparison Test: $p < 10^{-5}$). (B) Histogram quantifying the object preference of the population of units, according to the unit's maximum correlation index. The single-object PSTH that resembles mostly the multi-object PSTH results into the highest correlation index. Most units showed the highest correlation index when comparing the object B with the multi-object PSTH. (C) Time course of correlation indices calculated from 40 ms time windows of the PSTHs of each single-object PSTH correlated to the corresponding time window in the multiple-object PSTH. Before passing object A, the multi-object PSTH mostly resembled the object A PSTH. Thus, object A had the highest impact on the response pattern to the multiple-object sequence. After passing object A, the multi-object PSTH was mostly affected by object B. The letters "A" and "B" above the boxplots indicate the time windows where object A and object B led to higher correlation values, respectively. The letters "A" and "B" are temporally confined before and after passing object A, respectively. Kruskal-Wallis and Dunn's multiple comparison post hoc test; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Robustness of cortical and subcortical processing in the presence of natural masking sounds

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Status submitted

Abstract Processing of ethologically relevant stimuli could be masked by concurrently occurring irrelevant stimuli. Although behavioral adaptations, or jamming avoidance responses (JARs), which reduce the chance of masking have been reported, it is largely unexplored if the JARs facilitate neuronal processing of relevant stimuli. Here we describe JARs of the frugivorous bat *Carollia perspicillata* and tested neurophysiologically, if the JARs could facilitate neuronal processing of biosonar information. For orientation, bats emit high frequency echolocation calls and they often have to segregate and process their own calls and echoes in the presence of vocalizations from conspecifics. Our results show that the bats structure their calls into call groups, in noisy environments. Grouping the calls increases the discontinuity of sensory acquisition. Each echo of the group encodes similar information because of the high call during the emission of call groups. The latter makes some echoes expendable for the bats and interferences less dramatic. To be able to rely on such JARs, the increased call rates should not tremendously increase neuronal suppression. Thus, the robustness of neuronal tuning to an echolocation sequence (target) was tested in the absence and presence of biotic noise (masker). Neurophysiological results from the auditory midbrain and cortex show that the neuronal response to the target is still represented in the presence of the masker. Thus, the JAR reported in *C. perspicillata* could facilitate neuronal processing of ethologically relevant stimuli.

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(2) zur Durchführung der einzelnen Untersuchungen und Experimente

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Author Contributions

M.J.B., S.K. performed experiments. M.J.B. analysed data. M.J.B. wrote manuscript. M.J.B., F.G., M.K., and J.C.H conceived and directed the study. All authors discussed the results and commented on the manuscript.

Conflict of interest

The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.J.B. beetzjerome@gmail.com

Abstract

Processing of ethologically relevant stimuli could be masked by concurrently occurring irrelevant stimuli. Although behavioral adaptations, or jamming avoidance responses (JARs), which reduce the chance of masking have been reported, it is largely unexplored if the JARs facilitate neuronal processing of relevant stimuli. Here we describe JARs of the frugivorous bat *Carollia perspicillata* and tested neurophysiologically, if the JARs could facilitate neuronal processing of biosonar information. For orientation, bats emit high frequency echolocation calls and they often have to segregate and process their own calls and echoes in the presence of vocalizations from conspecifics. Our results show that the bats structure their calls into call groups, in noisy environments. Grouping the calls increases the discontinuity of sensory acquisition. Each echo of the group encodes similar information because of the high call during the emission of call groups. The latter makes some echoes expendable for the bats and interferences less dramatic. To be able to rely on such JARs, the increased call rates should not tremendously increase neuronal suppression. Thus, the robustness of neuronal tuning to an echolocation sequence (target) was tested in the absence and presence of biotic noise (masker). Neurophysiological results from the auditory midbrain and cortex show that the neuronal response to the target is still represented in the presence of the masker. Thus, the JAR reported in *C. perspicillata* could facilitate neuronal processing of ethologically relevant stimuli.

Significance Statement

Animals need to process and segregate behaviorally relevant stimuli from irrelevant ones. Here, we describe behaviorally and neurophysiologically how bats process biosonar signals in the presence of potentially masking vocalizations of conspecifics. We show that under noisy conditions, the bats increase the tendency of emitting echolocation calls in groups and decrease the call intervals within the call groups. The temporal changes of call emissions increase the discontinuity of biosonar information but could improve the continuity of neuronal echo information. The latter gets supported by data from the auditory midbrain and cortex. The increased sensory acquisition rate does not increase neuronal suppression and masked echo information could be compensated by relying on non-masked echo information.

Introduction

For understanding how nervous systems work, it is necessary to analyze neuronal processing of natural and ethologically relevant stimuli (1, 2). Since animals encounter a mixture of different stimuli, neuronal processing of ethologically relevant stimuli could be masked by the presence of irrelevant stimuli. Throughout this manuscript, we will refer to the relevant stimuli as “targets” and the irrelevant stimuli as “maskers". Although, many behavioral adaptations, also called jamming avoidance responses (JARs) have been reported to reduce or even avoid masking (3-8), it remains largely unexplored if neuronal processing of targets profit from the JARs. The present study reports JARs in the frugivorous bat *Carollia perspicillata* and tested if the JARs could facilitate neuronal processing of echolocation sequences in the presence of masking sounds.

Bats orientate acoustically by emitting biosonar calls that are reflected in surrounding objects (9-11). For computing the distance to objects, bats measure the time interval from call emission to echo arrival, also called echo delay. The echo delay decreases linearly with decreasing distances. Since bats need to integrate call and echo information, the computation of distances could be interfered by acoustic maskers from call onset to echo arrival (12). Neurons involved in distance processing, also called delay tuned neurons, integrate call and echo information to respond to certain echo delays (13-19). Thus, bats represent good animal models to test if JARs could facilitate neuronal processing of distance information. Neurophysiologically, we focused on the inferior colliculus (IC) and the auditory cortex (AC) because most data from delay tuned neurons were obtained by recording neuronal signals from these two brain regions.

Our behavioral results show that *C. perspicillata* adapts its call emission pattern in the presence of maskers. The bats increase the tendency of emitting grouped calls and they reduce the call intervals (CIs) within call groups. High stimulus rates usually evoke neuronal suppression (20-22) which has also been shown in *C. perspicillata* (23, 24). Thus, we tested if the increase in stimulus rate may also increase neuronal suppression which could impede target detection at the neuronal level. Our results show that in the IC, the neuronal suppression did not vastly increase and the neuronal tuning to the target was barely affected by maskers. In contrast, the suppression increased in the AC. But, target detection was higher in the AC than in the IC due to a higher neuronal selectivity in the AC. In summary, our results indicate that the JARs, reported here for *C. perspicillata*, could facilitate neuronal processing of the targets in the presence of maskers.

Results

Behavioral adaptations to acoustic masking during echolocation

In a flight room (Fig. 1A), the echolocation behavior from eight bats was monitored in the absence (training trials) and presence (test trials) of maskers. A wall, made out of foam, separated the room into two acoustically isolated sides. In training trials, under non-masking conditions, the hand released bats had to land on one out of two platforms at the end of the room (Movie S1). Two ultrasound sensitive microphones, each positioned behind a platform, recorded the call emissions. In test trials, under masking conditions, a masker was presented from one side of the room, whereas the contralateral side was silent. The masker represents a sequence of echolocation calls that was recorded during the first test trial from the tested bat (for details see methods). The bats preferred to land on the platform of the non-masking side (Paired t-test: $p = 0.01$; Fig. 1B; Movie S2). But, they could still echolocate and land on the platform at the masking side (Movie S3).

When comparing the call patterns emitted at the masking side during test trials with the ones emitted during training trials (exemplarily shown in Fig.1C), it becomes evident that the bats changed their emission pattern in the test trials. Minimum CIs decreased during test trials (Wilcoxon signed rank test: $p = 0.041$, Fig. 1D). Median CIs, maximum CIs, and the number of emitted calls did not differ between the two conditions (Wilcoxon signed rank test: $p > 0.05$, Fig. S1A, Fig. S1B). Behavioral studies reported that bats often emit groups of echolocation calls (*C. perspicillata* (25, 26), *Phylostomus discolor* (27), *Eptesicus fuscus* (28-35), *Myotis lucifugus* (36), *Tadarida brasiliensis* (37), and *Noctilio albivenris* (38)), a behavior that was also observed in the present study (signaled by black and red dots on top of each call pattern in Fig. 1C). During test trials, the bats did not change the relative amount of grouped calls (Fig. S1C; median = 59.7% under masking and 60.6% under non-masking; Paired t-test: $p = 0.79$). However, the bats increased the size of the call groups, indicated by the number of calls per call group (Fig. 1E; Fig. S1D). The bats emitted significantly more triplets under masking than under non-masking conditions (Wilcoxon signed rank test: $p = 0.046$). Trials in which quartets, quintets, and sextets were emitted were too few for statistical comparison. Though, note that the mean values of the grouping rates for quartets and sextets were higher, under masking than under non-masking conditions (quartet = 10^{-4} vs. 13^{-3}, sextet = 9^{-4} vs. 10^{-4}, for non-masking vs. masking). Additionally, the bats decreased the median CI within the call groups from 25 to 22 ms (paired t-test: $p = 0.02$; Fig. 1F) which results into acoustic rates of 90 Hz (when considering call and echoes).

Masker effect on single-unit responses in the inferior colliculus and auditory cortex

Acoustic rates higher than 40 Hz usually evoke neuronal suppression which is also the case for the IC (23) and AC (24, 39) of *C. perspicillata*. Thus, the bat may only profit from the previously described JAR if the neuronal response to the target is not suppressed in the presence of the masker. The target was an echolocation sequence that mimicked a stimulus scenario in which the bat flies towards an object. Robustness of neuronal tuning to the target was tested by mixing the target with two different maskers (for details see methods) which leads to "mixture conditions". The "single animal mixture" simulates a situation where two bats fly in the same room. "The colony mixture" simulates a situation in which many bats fly together. Note that the single animal masker condition resembles the situation that bats had to encounter in the behavioral experiments.

Respectively, neuronal activity from 49 collicular and 72 cortical single-units from six and nine bats was recorded. In response to the target, cortical units responded selectively to particular call-echo elements (Fig. 2B, right column). The latter stands in contrast to the collicular units (see example in Fig. 2B, left column) which responded to almost each acoustic event in the target echolocation sequence (see also Fig. S2). Both collicular and cortical units responded sparsely to the single animal masker (red raster plot and PSTH in Fig. 2B-C). The cortical unit showed a strong onset response which is common in the AC and is independent of the echo delay (24). Therefore, the initial 150 ms of the cortical responses were excluded from the analysis. The neuronal firing pattern in response to the single animal mixture (black raster plots and PSTHs in Fig. 2B-C) was more similar to the response to the target than to the single animal masker. The latter was quantified by correlation values between the corresponding PSTHs (Wilcoxon signed rank test: $p < 10^{-5}$; black crosses in Fig. S4A and Fig. S4B). In response to the single animal mixture, the cortical unit was selectively tuned to an echo delay of 7 ms (black raster and PSTH in the right column of Fig. 2B-C). In addition, between 200-400 ms after stimulus onset (violet arrowhead in Fig. 2C), a neuronal response was detected that was neither present in response to the target nor to the masker. This facilitation is caused by a stimulus integration of the target and the masker and may represent a neuronal correlate of jamming. Collicular and cortical neurons responded stronger to the colony than to the single animal masker (red raster plot and PSTH in Fig. 2). However, note that the cortical neurons responded more selectively to particular segments of the masker than the collicular neurons. The interference from the colony masker was higher than the one resulting from the single animal masker. The latter is indicated by higher similarities between the responses to the colony mixture and the colony masker than the ones between the colony mixture and the target (Paired t-test: $p < 10^{-5}$; green crosses in Fig. S4A and Fig. S4B). The response pattern to the target was only poorly detectable in the colony mixture response (Fig. 2E).

To quantify the effects of the masker on distance processing, we compared the “median echo-delays” in response to the target and the mixture. Based on the spike times, each spike was associated to a particular echo-delay of the target. The median of those delays represent the “median delay” of the unit. In response to the single animal mixture, the median delay did not change for 28.6% and 33% of the collicular and cortical units, respectively (black bars in Fig. 3A and 3B at Δ median delay = 0). In the AC, the colony masker evoked a stronger median delay shift than the single animal masker (Wilcoxon signed rank test: $p < 10^{-5}$; Fig. 3C). Similar results were obtained by considering the “best delay”, represented by the echo delay evoking the peak in the PSTH, instead of the median delay (Fig. S4C-4E). Note that the median delay usually shifted towards longer delays in the mixture situation. The median delay was more robust and shifted less in the IC than in the AC (Kruskal-Wallis test and Dunn’s multiple comparison post-hoc test: $p < 10^{-5}$; Fig. 3C).

To quantify whether the target is encoded in the responses to the mixtures, we calculated d’ values with equations from the signal detection theory for each unit (IC Fig. 3D; AC Fig. 3E, (40, 41)). D’ values represent differences of the spike rate obtained in response to the masker and the mixture. High d’ values either arise from a response to the target or from a response caused by an integration of masker and target. The time course of d’ values was obtained by calculating d’ values for each call-echo element of the target (for details see methods and Fig. S3). To exclude spike rate differences that putatively come from integration of masker and target, we considered only d’ values from call-echo elements that evoke a spike rate of at least 50% of the maximum spike rate in response to the target. The sum of these d’ values represent the cumulative d’ for each unit. High cumulative d’ values indicate strong responses to the target in the mixture condition. The data showed that cumulative d’ values were higher for the single animal than for the colony masker (Wilcoxon signed rank test: $p < 10^{-5}$; Fig. 3F) and they were also higher in the AC than in the IC (Kruskal-Wallis test and Dunn´s multiple comparison post-hoc test: $p < 10^{-5}$; Fig. 3F). We got similar results when considering all bins of the PSTH for calculating cumulative d’ values (Fig. S4F-S4H). To summarize, our data shows that there exist different strategies for information representation in noisy environments along the colliculo-cortical axis. Masker induced median delay shifts are weaker in the IC than in the AC. At the same time, target representation, as calculated by d’ values, is higher in the AC than in the IC.

Masker effect on cortical target-distance map

In *C. perspicillata*'s AC, delay tuned neurons are topographically organized along the rostro-caudal axis (10, 42). Positioning multi-electrodes along the topographic gradient allows simultaneous characterization of long and short delay tuned neurons at caudal and rostral positions, respectively ((24, 43), Fig. 4A). We analyzed neuronal tuning in twelve cortical maps, eight from the left and five from the right cortex. Response patterns from two representative cortical maps are shown in figure 4B-4F and S5A-S5E. The topography was more distinct in response to the target (Fig. 4B, S5A) than to the single animal (Fig. 4D; S5C) and the colony mixture (Fig. 4F; S5E).

The median delays plotted against the electrode position give an overview of the topographic changes induced by the masker (Fig. 4G; S5F). In the presence of the colony masker, the topographic maps shifted towards longer delays than in the absence of the masker. Changes in neuronal tuning indicated by changes in delay tuning, response strengths at best delay, and cumulative d' were independent of the electrode position (Fig. S5G-S5J).

The direction of the topographic gradient can be read out by the sum of the median delay slopes (Σ slopes; Fig. 4H). Negative Σ slopes signal a decrease of median delay from caudal to rostral and vice versa. In response to the target, the median delays decrease from caudal to rostral 9 out of 12 maps (target maps). Median delays from the three remaining target maps did not change along the rostro-caudal axis. Five single animal and three colony mixture maps, changed their direction of the gradient and had positive Σ slopes.

The smoothness of a topographic map is represented by the absolute slopes between adjacent electrodes. Abrupt delay shifts result in high absolute slopes, or rough topographic maps. Subtle delay shifts result in low absolute slopes, or smooth topographic maps. To identify whether mixture maps were rougher or smoother than target maps, the sum of the absolute slopes (Σ abs slopes) in response to the target was subtracted from Σ abs slopes in response to the mixture (Fig. 4I). Positive values, along the y-axis, indicate a smoother map in response to the mixture than to the target and vice versa. Map roughness increased in 58% (black numbers in Fig. 4I) and 67% (green numbers in Fig. 4I) of the maps in the single animal and colony mixture, respectively. Rougher maps are partially explained by an increase in the delay range that the map covers. The latter is indicated by a linear correlation between the delay range and the absolute slope (Pearson: $r = 0.79$, $p < 10^{-5}$, Fig. 4I). In most cases, an increase of the delay range is a result of increasing the maximum delay instead of a decrease in the minimum delay (Fig. 4J). Cumulative d' values were calculated and plotted for each map (Fig. 4K). The d' values for the maps were significantly higher in the single animal masker than in the colony masker, indicating a higher target detection ability at the map level in the single animal mixture condition (Paired t-test: $p = 0.005$).

Discussion

Many JARs have been reported in different species (3, 7, 8). Generally spoken, acoustic signals are modified by the animals to make them distinct from the maskers. The validity of the JARs with respect to a putative facilitation of neuronal processing of targets remains largely unexplored. In the present study, *C. perspicillata* shows a JAR that is based on controlling the time points of call emission. Such JARs have been discussed in different animals, like crickets (44), frogs (45, 46), electric fish (47), birds (48, 49), bats (27, 37, 50, 51), and monkeys (52). In response to the maskers, *C. perspicillata* emits tight groups of calls. The fact that 60% of the calls were emitted in groups, independent from the presence of the masker, shows the importance of emitting call groups during echolocation. In bats, the emission of call groups has been associated with a high complexity of orientation tasks (28, 31-34, 37, 38). Behavioral results in the frugivorous bat *Phyllostomus discolor* also show an increase of call grouping in the presence of maskers (27), corresponding to the present results from *C. perspicillata*.

How could call grouping facilitate echolocation in the presence of maskers? For illustration, we consider the detection of two different target sequences (Fig. 5). Echolocation calls from the first target are equally distributed (non-grouped sequence; Fig. 5A) and the calls of the second target are grouped into triplets (grouped sequence; Fig. 5B). According to a median CI of 22 ms (red boxplot in Fig. 1F) and an average flight speed of 2.5 m/s (26), a triplet carries distance information from ± 11 cm. Thus, the call-echo elements, or functional units of a triplet, encode echo delays in the range of ± 1ms. If no functional unit gets masked, then the non-grouped sequence convey a higher spatial resolution (1 ms) than the grouped sequence (3 ms). However, if functional units get masked then two functional units from a triplet of the grouped sequence could get masked without losing much delay information. In contrast, the loss of delay information might be more dramatic if functional units get masked in the non-grouped sequence. Additionally, the maximum distance that the bat has to cover without delay information (maximum blind spot) is longer in the non-grouped than in the grouped sequence (in our example, 63 cm for the non-grouped and 51 cm for the grouped sequence). In summary, grouping the calls more tightly increases the discontinuity of echo information but it could increase the continuity of preserved delays (compare the continuity of preserved delays in (A) and (B)).

At the neuronal level, call groups and maskers increase the acoustic rate which could potentiate neuronal suppression (24, 43). Neuronal suppression is usually weaker at subcortical than at cortical areas (20, 23, 53). This may explain, why the maskers tend to induce stronger delay tuning shifts in the IC than in the AC. In the AC, delay tuning was usually shifted towards longer delays which could be based on two aspects: i) In noisy environments, the cortex is biased to process echo information from distant objects, like conspecifics. The latter is especially important for tracking flight trajectories of conspecifics for avoiding collisions. ii) The risk of interference is highest at long delays because the functional units span a

longer duration than the ones at short delays (Fig. 5). Thus, delay tuning shifts towards long delays reflect jamming effects.

The target representation in response to the mixture was computed by d' values that have been used in previous studies (40, 41). Corresponding to the high d' values in the AC, target detection in the mixture situation was higher in the AC than in the IC. This result could be explained by the differences of suppression strengths that affect the neuronal selectivity in both brain areas (54). In contrast to collicular neurons that usually respond to each acoustic event of an echolocation sequence (23), cortical neurons respond selectively to certain acoustic events (24). The increased selectivity makes the AC less sensitive to the maskers which results in higher d' values than in the IC (compare responses to colony masker in Fig. 2D). The fact that the increase of neuronal selectivity along the ascending auditory pathway could be correlated with an improvement of target detection in a noisy situation is supported by findings in frogs. The ability of target detection (55) and the neuronal selectivity (4, 56) increases from the auditory nerve to the IC of frogs.

Note that during the neuronal recordings, the animals were passively listening to echolocation sequences. Attentional effects, binaural effects, directionality of the stimuli and of hearing could further facilitate signal processing under noisy conditions (4, 29, 54, 57, 58).

In summary, the present results indicate that in noisy environments, *C. perspicillata* increases the discontinuity of echo information by emitting tightly grouped calls. Each functional unit of the group encodes a similar echo delay which makes some echoes expendable for the bat. Erroneously encoded distance information could get updated at neuronal level. At the neuronal level, moderate delay tuning shifts in the IC and high d' values in the AC indicate that target information is still encoded in the presence of the masker. Thus, the JARs, found in *C. perspicillata*, could potentially facilitate neuronal processing of natural and ethologically relevant stimuli.

Methods

Animals

Behavioral and electrophysiological experiments were conducted in eight (1 female, 7 males) and 15 female bats (9 for auditory cortex and 5 for inferior colliculus) of the species *Carollia perspicillata*. All bats were bred in a colony at the Institute for Cell Biology and Neuroscience (Frankfurt University). The experiments comply with all current German laws on animal experimentation and they are in accordance with the Declaration of Helsinki. All experimental protocols were approved by the Regierungspräsidium Darmstadt (experimental permit # F104/57).

Behavior

Behavioral experiments were performed in a wooden flight chamber (Movie 1-3; Fig. 1A; length: 4 m; width: 1.4 m; height: 2 m). A wall, made out of foam, separated the room into two sides, each side measuring 2.5 m x 0.65 m x 2 m. A landing platform (20 x 20 cm), made out of metal mesh, was positioned in one of the walls in each side of the room. Behind each metal mesh, one speaker (Neo CD 1.0 Ribbon Tweeter; Fountek Eelctronics, China) and one ultrasound sensitive microphone (Avisoft Bioacoustics, Germany) were installed. The microphones had a sensitivity of 50 mV/Pa and an input-referred self-noise level of 18 dB SPL. Each microphone was connected to a sound acquisition system (one microphone to an UltraSoundGate 116 Hm mobile recording interface and the second microphone to an UltraSoundGate 116 Hb mobile recording interface, + Recorder Software, Avisoft Bioacoustics, Germany) for sound digitalization at 333 kHz (16 bit precision).

During the training trials, call emissions of hand released bats were acoustically recorded with the microphones. The flight behavior was monitored with a webcam (500 SX, Manhattan, USA) placed above the starting point and with a frame rate of 30 Hz. During test trials, one speaker produced playback stimuli resulting in a potentially masking side. The speaker of the contralateral side remained silent (non-masking side). Masking and non-masking sides were randomly selected. The playback stimuli represented repetitions of a representative biosonar call that was recorded during a training trial from the tested animal. The biosonar call was repeated in groups of five (one animal), ten (four animals) or twenty calls (three animals) with within group call intervals of 15 ms and in between group intervals of 35 ms. The within group call intervals of 15 ms lies in the range of the minimum pulse intervals produced by *C. perspicillata* (26). Behavioral data from bats that were stimulated with call groups consisting of five, ten, or twenty calls were not different and therefore were grouped together for data analysis. Acoustic stimuli were generated with a sampling rate of 384 kHz with an Exasound E18 sound card (ExaSound Audio

Design, Canada), and sent to an audio amplifier (Rotel power amplifier, RB-850, USA). The stimuli were played with a sound pressure level of 80-90 dB re 20 µPascal (dB SPL).

The call emissions were recorded by the microphones and a segment of two seconds was analyzed from each trial. The segment was chosen such that the echolocation calls with the highest amplitude during the trial were considered. This reduced the risk of missing echolocation calls whose amplitude are too low to be recorded. The analysis segments from test trials were chosen from recordings conducted while the animals flew at the masking side. In total, 48 segments from eight animals were analyzed. Twenty-four segments (3 per animal) were recorded during the training and another 24 were recorded during the test trials. Call emission patterns during test and training trials were compared pairwise, meaning that three pairs of "test" and "training" trials were compared for each animal.

For data analysis, the time points of call emissions were manually tagged in the software Avisoft SAS Lab Pro (Avisoft Bioacoustics, Germany). These time points were later used for the remaining analysis done in Matlab 2014 (MathWorks, USA). Call groups were defined according to the criterions formulated by (28). First an "island criterion" defines temporally isolated call groups. The island criterion is fulfilled when the preceding and following call intervals of a call group are 20% longer than the call intervals within call groups. If the island criterion is fulfilled a second criterion, the so called "stability criterion", defines the size of the call groups indicated by the number of calls belonging to a group. The stability criterion is fulfilled if the call intervals within call groups are stable with a 5% tolerance. Note that doublets, i.e. call groups containing two calls, can only be defined according to the island criterion. For determining triplets, quartets, quintets or sextets, both criteria had to be fulfilled. The behavioral analysis considered doublets to sextets, because sextets represent the longest call groups reported in *C. perspicillata* (25).

Stimuli for electrophysiology

To study neuronal responses, we used two types of "maskers" and one "target" stimuli. The target was an echolocation sequence recorded from a pendulum flight simulating an approach flight (24). In a previous article, we had already shown that the target evoked reliable neuronal responses in high-frequency tuned neurons of the IC and AC of *C. perspicillata* (23, 24). Note that in the target, the echo delays decreased from 23 ms at the first, to 1 ms at the last call-echo element. The recording and preparation of the echolocation sequence was explained in detail, previously (24). Briefly, the acoustic signals were recorded using a pendulum paradigm (59), in which the bat was positioned in a pendulum mass and it was swung towards an acrylic glass wall (24). During the swing, the bat emitted sequences of echolocation calls that were recorded, together with their echoes, with the aid of an ultrasound sensitive

microphone attached to the pendulum (Avisoft Bioacoustics, Germany). The SPL of the acoustic events of the target echolocation sequence varied between 36-77 dB SPL.

As masker stimuli three different sound sequences were used. Two of them were defined as “single animal masker” and the remaining one was described as “colony masker”. The single animal maskers consisted of echolocation calls coming from one individual. One of the single animal maskers contained 33 calls that were recorded with the pendulum paradigm. Echoes were manually deleted from the sequence. In other words, except the missing echoes, the single animal masker was natural in the sense of spectral, temporal, and intensity parameters. The SPL of the calls varied between 62 and 82 dB (median = 71 dB SPL). The second single animal masker was defined as semi-natural because the level, spectrum and temporal properties of the natural echolocation calls that composed this masker were stable throughout the stimulation. The echolocation call, used as building block for the second single-animal masker was repetitively presented to the animals in form of quartets. The CIs within call groups was 23 ms and 83 ms between groups. The single animal masker simulates an acoustic environment where two bats echolocate close to each other. Data obtained with both single animal maskers were comparable, and therefore they were grouped together for analysis.

In comparison to the single animal masker, the colony masker contained more acoustic events, including calls, echoes, and communication calls from a colony of *C. perspicillata* with 150 animals. The colony masker was recorded with an ultrasound sensitive microphone that was held for 30 seconds inside of the colony room of the facility. A segment of 1.34 seconds of the recording was used as colony masker. The masker contained more than 200 acoustic events that partially overlapped in time and the SPL of the acoustic events ranged from 43 to 81 dB (median = 63 dB SPL). Due to temporal overlap, it was impossible to measure the exact number of acoustic events in the colony masker. The colony masker reflects a natural acoustic environment that *C. perspicillata* has to face in the roosts. The “single animal mixture” and the “colony mixture” represent stimuli that were obtained by adding the target stimulus to the single animal and colony maskers, respectively. Stimuli were presented fifteen times.

Electrophysiological recordings

Electrophysiological recordings took place in a sound-proofed and electrically-shielded chamber. Recordings from the IC were focused on the central nucleus of the left IC whose position was determined based on the tonotopic arrangements of the recorded units. Neuronal signals from the AC were recorded in the left (n = 8) and right (n = 5) hemispheres. For the surgery, the bats were anaesthetized with a subcutaneous injection of a mixture of ketamine (10 mg/kg Ketavet, Pharmacia GmbH, Germany) and xylazine (38 mg/kg Rompun, Bayer Vital GmbH, Germany). A longitudinal midline incision was made through the skin overlying the skull. Muscle tissue, covering dorsal and temporal parts of the skull, was

removed. For cortical recordings, a craniotomy was made over the high frequency area of the brain, to gain access to auditory neurons. For subcortical recordings, a craniotomy above the sulcus separating the cerebrum and cerebellum, gave access to the IC. For the fixation of the bat's head, a custom-made metal rod (1 cm length, 0.1 cm diameter) was glued onto the skull using dental cement (Paladur, Heraeus Kulzer GmbH, Germany). Each bat was used for chronical recording sessions that lasted several hours over a period of two weeks. At the day of recording, the animals were lightly anaesthetized with a small dose (0.03 mL) of ketamine/xylazine mixture diluted in sodium chloride. This small dose allowed us to place the animal in the recording setup and to position the electrodes either in the AC or IC. Neural data acquisition started as soon as the animals woke up from anesthesia. That the bats had woken up was assessed by spontaneous and auditory evoked movements of the pinna, mouth, and nose-leaf. These movements were first visible about one to two hours after the initial dose of anesthesia.

For cortical recordings, two electrode types were used. (i) Commercially available micro-electrode arrays with 16 recording electrodes organized in 2 x 8 (MicroTargets for Life Science, USA). A reference electrode with an impedance of 10 kΩ was placed adjacent to the recording electrodes. Reference and recording electrodes were made out of tungsten whereas a silver wire placed on the cortical surface of the frontal cortex was used as ground. Each recording electrode had an impedance of 2 MΩ (as reported by the manufacturer). The arrays had an electrode and row spacing of 250 µm. (ii) Custom-built glass electrode arrays of up to 8 channels organized in a single row. Glass electrodes (resistance 1-10 MΩ when filled with 3 mol/L KCl) were pulled from borosilicate capillaries (GB120F-10, Science Products, Germany) with a Flaming/Brown horizontal puller (P97, Sutter, USA) and they were glued together in a fan-shape pattern, ensuring an electrode tip spacing of 250 µm. For IC recordings, single glass electrodes were used with the same specifications as for the glass electrodes of the custom-built glass electrode arrays.

A wireless multichannel recording system (Multi Channel Systems MCS GmbH, Germany) was used for data acquisition at a sampling rate of 20 kHz per channel and 16 bit precision. Action potentials were filtered using a 2^{nd} order Butterworth band-pass filter, with cutoffs between 300-3000 Hz.

Spike-Data Analysis

Spike detection was based on spike amplitude relative to recording noise level. The spikes were sorted based on the first three principal components of the spike waveforms and they were clustered automatically using the "KlustaKwik" algorithm (60). Only the cluster containing the largest amount of spikes was used for analysis.

Neuronal data from the AC comprised 72 spike-sorted single-units that were recorded from twelve cortical maps. Between three and twelve units were recorded simultaneously (median = 6). Twenty units

were recorded with the commercially available micro electrode arrays from Microprobes and 52 units were recorded with the custom-made glass electrode arrays. Neuronal data from the IC comprised 49 spike-sorted single-units that were sensitive to high frequencies (> 40 kHz).

Data analysis was based on post-stimulus time histograms (PSTHs) constructed with a binsize of 5 ms and 2 ms for cortical and collicular data, respectively. Different binsizes between collicular and cortical units were used because collicular neurons fire temporally more precise than cortical ones (18). The initial 150 ms of the cortical response were not considered because of strong stimulus independent onset responses (24, 43). Delay tuning to the target stimulus was assessed by assigning each spike, according to its occurrence, to a specific echo delay of the echolocation sequence used as stimulus. The assignment of each spike to a specific delay allowed us to reconstruct delay tuning curves. The best delay is defined based on the call-echo element eliciting the strongest response i.e. the largest number of spikes. The median delay was calculated by measuring the median time of occurrence of the evoked spikes. The median time point was then assigned to the preceding call-echo element whose echo delay represents the median delay. In comparison to the best delay, which reflects the maximum response only, the median delay calculation considers each elicited spike. A response to certain echo-delays was considered to occur if the neuronal response to the target sequence was at least as strong as 50% of the maximum response observed (i.e. the response strength at the best delay).

For quantifying the preservation of the response to the target in the mixture situation (target detection), we calculated unit specific cumulative d' values (Fig. S3). First we determined median d' values from the response window to each call-echo element of the target.

$$d'^2_i = \frac{(mix_i - m_i)^2}{0.5\,[\sigma(mix_i)^2 + \sigma(m_i)^2]}$$

In the equation, mix_i and m_i represent the median spike rate in response to the call-echo element in the mixture and masker situation, respectively. Thus, high spike rate differences between the response to the mixture and masker result in high d' values. These differences are due to the presence of the target in the mixture and may either arise from responses to the target or from an integration of the target and masker stimulus. A neuronal response arising from stimulus integration represents a neuronal correlate of jamming. To determine the target detection in the mixture situation it is necessary to exclude high d' values arising from jamming. Therefore, we calculated a cumulative d' for each unit by considering only d' values from call-echo elements that elicited a neuronal response in response to the target stimulus *i*. Cumulative d' values were calculated according to the following formula:

$$cumulative\ d' = \sqrt{\sum_i d'^2_i}$$

The higher the neuron's cumulative d', the higher is its target detection in the mixture situation. The quantification of target detection based on d' have been successfully performed in previous studies (40, 41).Data analysis was done in Matlab 2014 and statistics in GraphPad Prism 5 (GraphPad Software, USA; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$).

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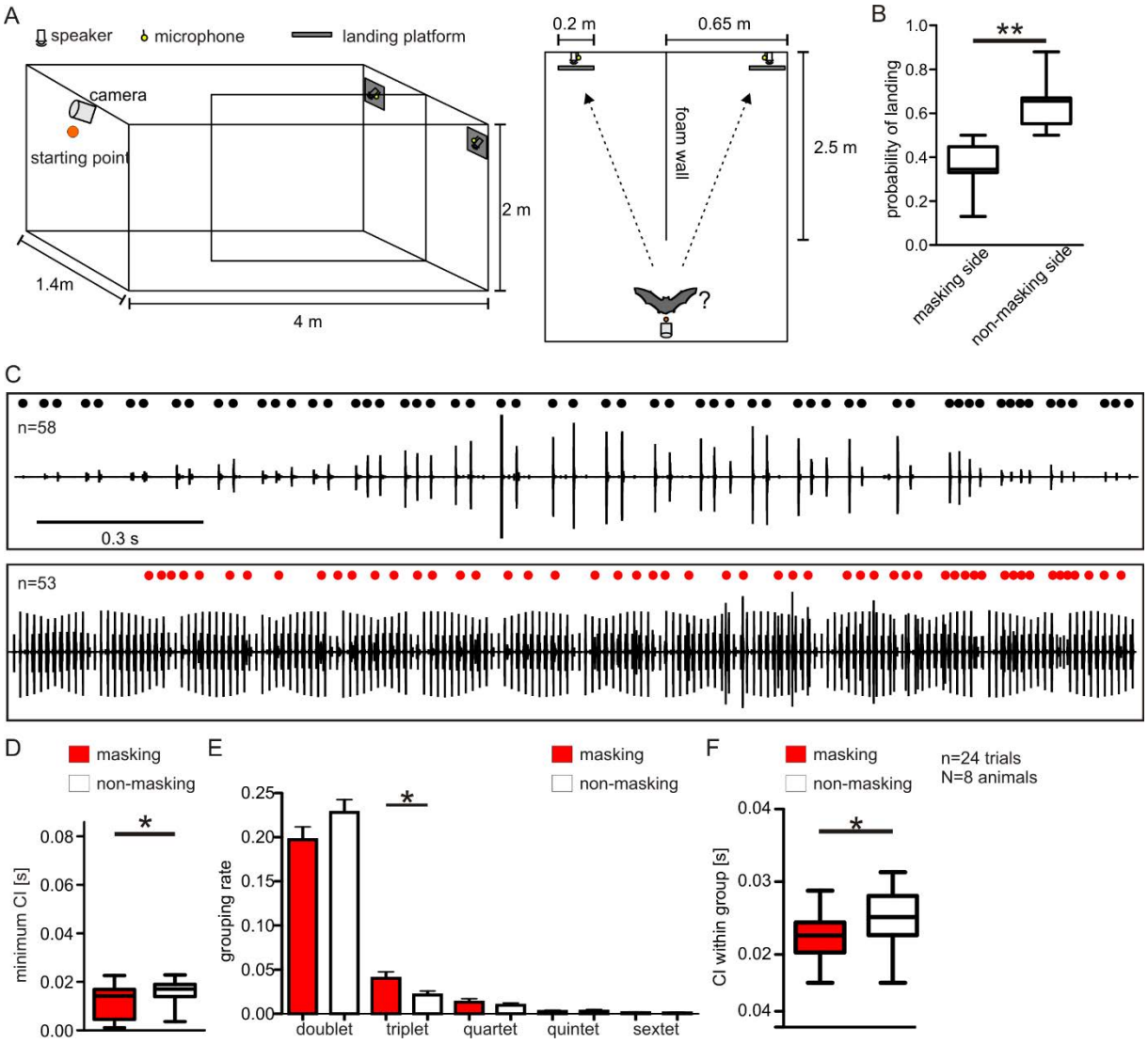


Figure 1 Behavioral adaptations of *C. perspicillata* when echolocating in noisy areas.
(A) Schematic lateral (left) and top view (right) of the flight room. (B) Boxplots demonstrate the rate of landing on the platform at each side. (C) Representative oscillograms illustrate the call patterns of one bat in the absence (top; non-masking condition) and presence (bottom; masking condition) of maskers. Respectively, black and red dots represent time points of call emission during non-masking and masking conditions. *n* indicates the amount of emitted calls. Note that the lower oscillogram contains the masker in addition to the emitted calls. (D) Minimum call intervals (CIs) during masking and non non-masking conditions. (E) Histogram shows the relative amount of call groups containing two (doublet), three (triplet), four (quartet), five (quintet) and six (sextet) calls in the non-masking and masking condition. (F) Boxplots illustrate the CIs within call groups.

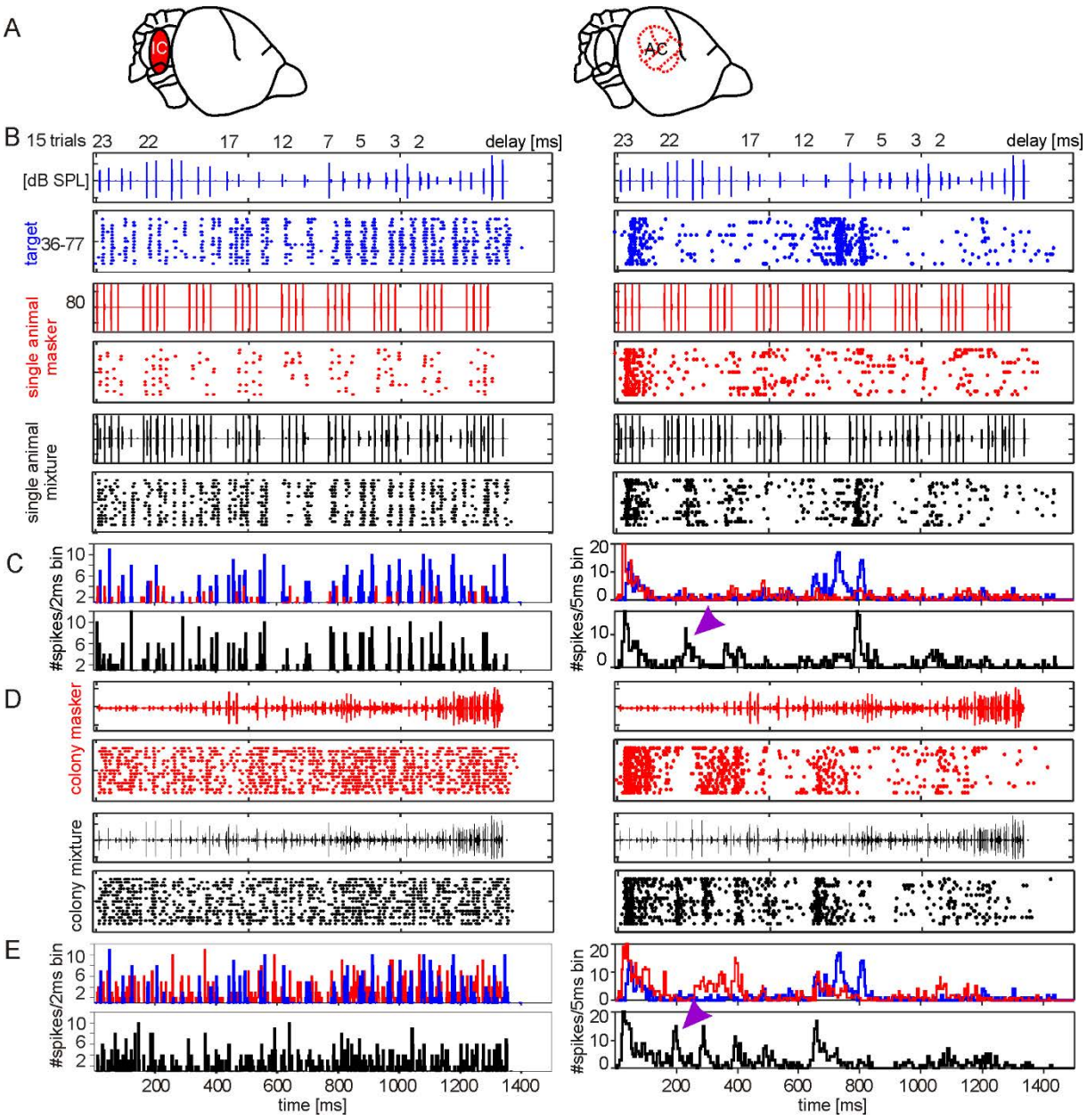


Figure 2 Neuronal responses of a collicular (left column) and a cortical (right column) unit.
(A) Schematic lateral view on *C. perspicillata*'s brain. Recorded brain areas are highlighted (inferior colliculus (IC); auditory cortex (AC)). Raster plots (B, D) and PSTHs (C, E; binsize = 2 ms for collicular and 5 ms for cortical unit) show the neuronal activity of a representative collicular and cortical unit in response to the target (blue raster plot in A, blue PSTHs in B and C), to the single animal masker (red raster plot in B, red PSTH in C), to the colony masker (red raster plot in D, red PSTH in E), to the single animal mixture (black raster plot in B, black PSTH in C), and to the colony mixture (black raster plot in D, black PSTH in E). Oscillograms of the stimuli are indicated above each raster plot. Violet arrowheads point to neuronal responses elicited by a stimulus interference of the target and masker.

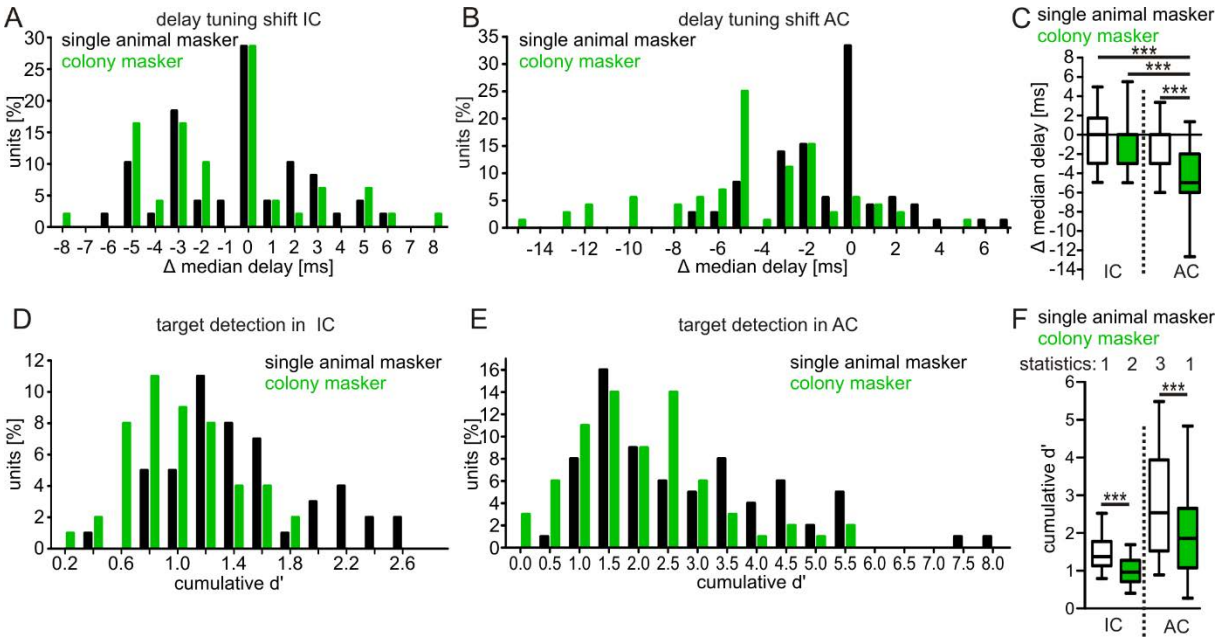


Figure 3 Robustness of neuronal processing in the presence of masker stimuli.
Histograms show the median delay shifts between target and mixture response in collicular (A) and cortical (B) units. Respectively, negative and positive values indicate delay shifts towards longer and shorter delays in response to the mixture condition. (C) Boxplots summarize the median delay shifts in the inferior colliculus (IC) and auditory cortex (AC). (D, E) Histograms show the target detection, indicated by cumulative d' values, at neuronal level in the mixture situation in the IC (D) and AC (E). (F) Boxplots summarize the cumulative d' values calculated for the IC and AC.

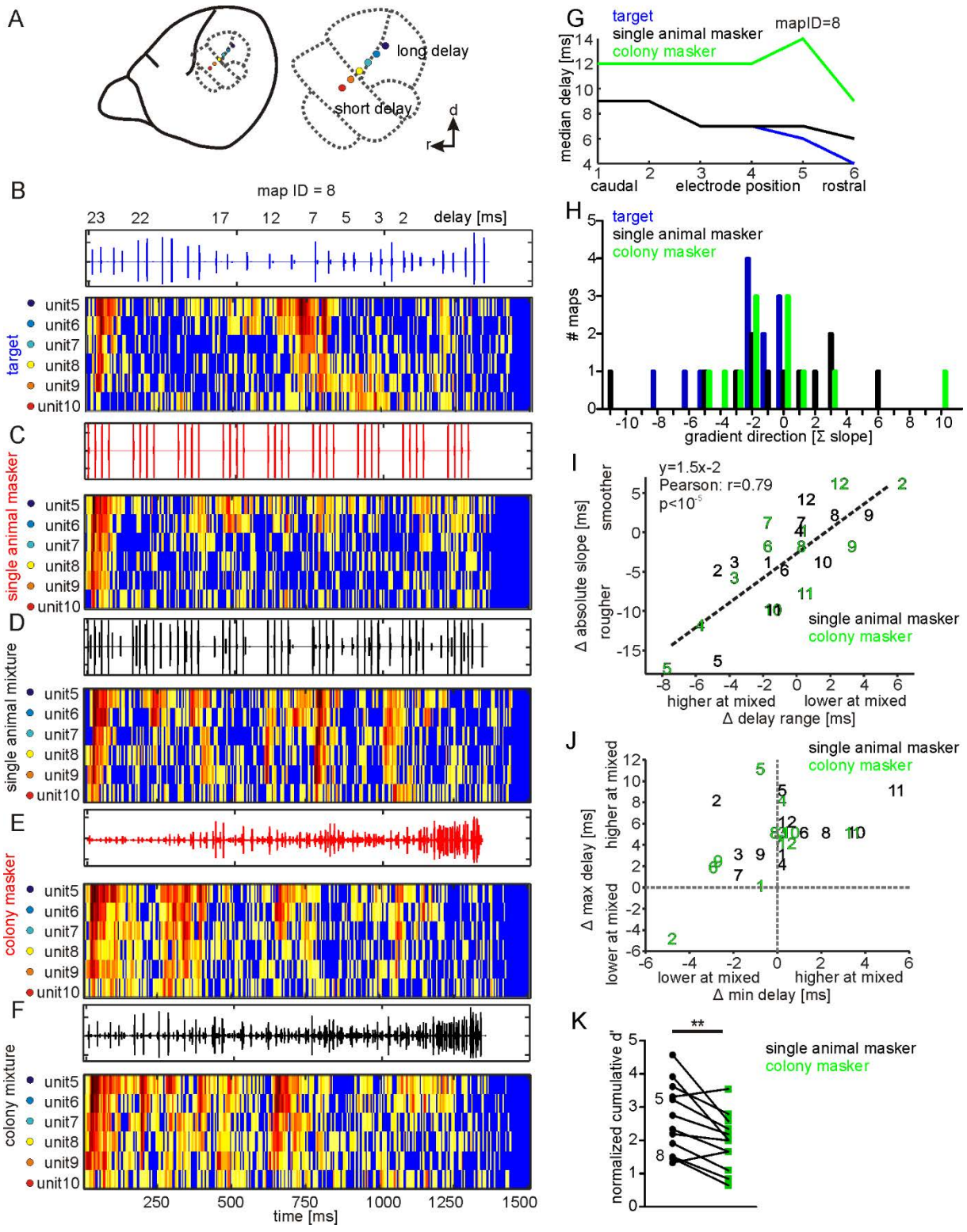

A
long delay
short delay
d
r
B
map ID = 8
23 22 17 12 7 5 3 2 delay [ms]
target
unit5
unit6
unit7
unit8
unit9
unit10
C
single animal masker
D
single animal mixture
E
colony masker
F
colony mixture
250 500 750 1000 1250 1500
time [ms]
G
target
single animal masker
colony masker
mapID=8
median delay [ms]
caudal
electrode position
rostral
H
maps
gradient direction [Σ slope]
I
y=1.5x-2
Pearson: r=0.79
p<10^-5
smoother
rougher
Δ absolute slope [ms]
higher at mixed
lower at mixed
Δ delay range [ms]
J
higher at mixed
lower at mixed
Δ max delay [ms]
Δ min delay [ms]
K
**
normalized cumulative d'

Figure 4 Robustness of chronotopy in the presence of masker stimuli.
(A) Schematic lateral view on *C. perspicillata*'s brain and a magnification of the auditory cortex (dashed lines). (B-F) Color-maps represent neuronal activity (binsize = 5 ms) from a cortical map in response to the target (B), single animal masker (C), single animal mixture (D), colony masker (E), and colony mixture (F) condition. Each row represents a unit. Colored dots indicate electrode positions corresponding to the color code from (A). (G) Median delays in response to the target (blue), single animal mixture (black), and colony mixture (green) are plotted against the electrode positions for the example map. (H) Histogram shows the gradient direction of the maps. Negative values indicate a decrease from long to short delays along the caudo-rostral extent and vice versa. (I) For each map, the absolute median delay slopes and the delay range that the map covers were subtracted from each other and plotted for each map. Each number represents a map identifier (ID). The linear regression shows that the map roughness partially depends on the delay range. (J) The change in maximum delay is plotted against the change in minimum delay for each map and mixture condition. (K) Cumulative map d' values are plotted for both mixture situations. The map-IDs of the example maps from (A-F) and Fig. S4 are indicated adjacent to the black data points.

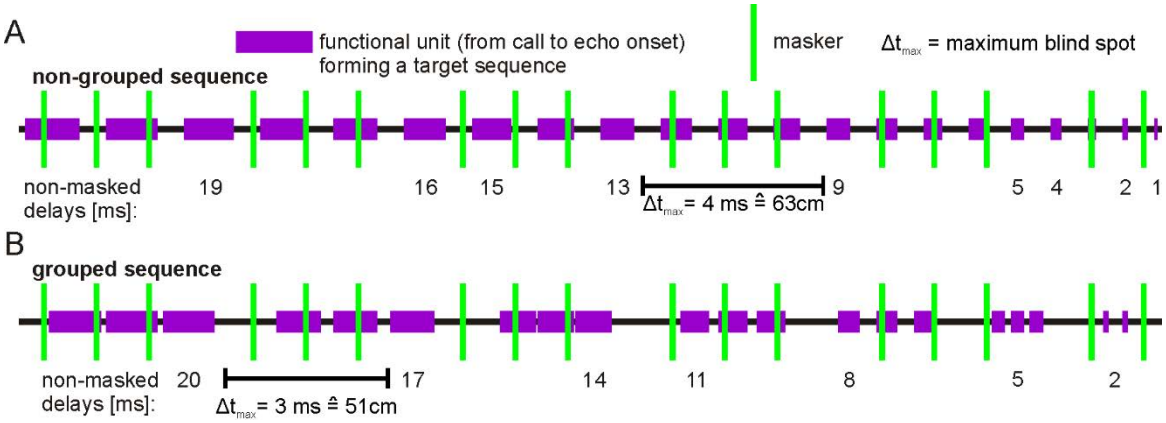


Figure 5 Grouping calls makes distance processing more robust against maskers.
Two schematic echolocation/target sequences are presented in violet. Each violet bar represents a functional unit spanning the time from call to echo onset. Both sequences contain 21 functional units, but they have different time structures. In (A) the calls are equally distributed (non-grouped sequence) and the encoded echo delays range from 21 to 1 ms in 1 ms steps. In (B) the calls are grouped into triplets where each functional unit of a triplet encodes a similar delay (< ± 1 ms). The delay information encoded by a functional unit gets masked if a masker occurs during the functional unit. Non-masked delays are indicated below each sequence. Δt_{max} represents the maximum blind spot that the bat would perceive in case of the masker presence.

Supporting information

Movie S1 Exemplarily training trial. The bat was hand released from a starting point and it landed on the left platform.

Movie S2 One test trial showing that the bat avoids to land on the platform at the masking side. Masking stimuli were presented from the left side.

Movie S3 One test trial showing that the bat could still land on the platform at the masking side. Masking stimuli were presented from the right side.

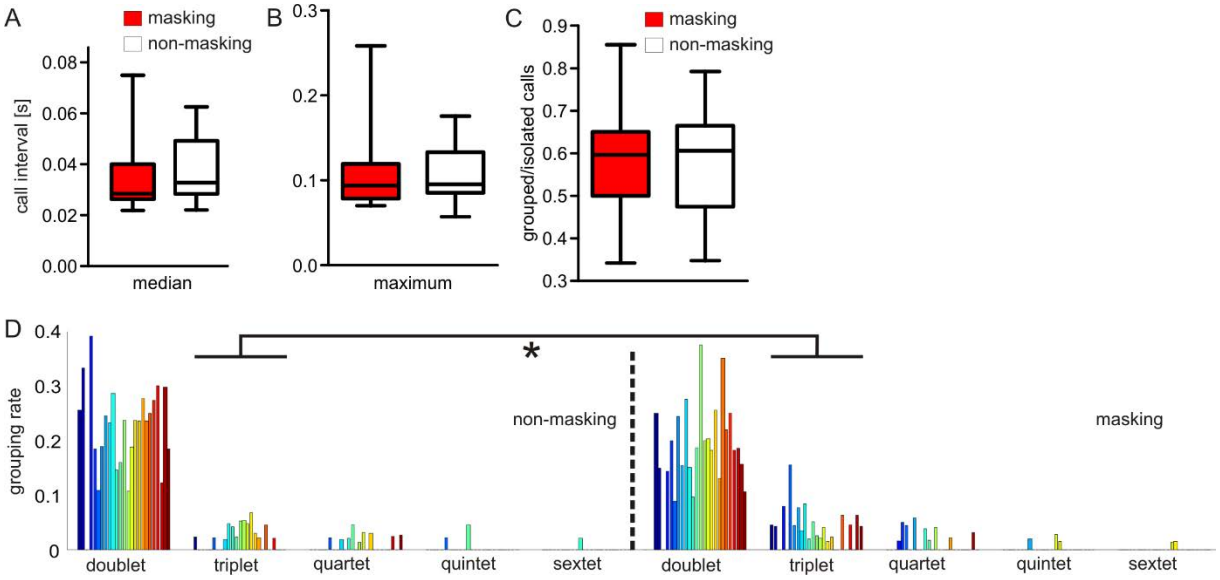


Figure S1 Behavioral adaptations of *C. perspicillata* when echolocating in noisy environments.
(A) Median and (B) maximum Call intervals were not affected by playback stimuli. (C) Relative amount of calls emitted in groups did not differ significantly between masking and non-masking conditions. Note that most calls (about 60% in both conditions) were emitted in groups. (D) Histograms showing the relative amount of call groups consisting of two (doublet), three (triplet), four (quartet), five (quintet) and six (sextet) calls in the non-masking and masking condition. Each color represents one pair of trials (24 pairs, 3 pairs each animals) of one animal. Under masking conditions more triplets were emitted than under non-masking conditions.

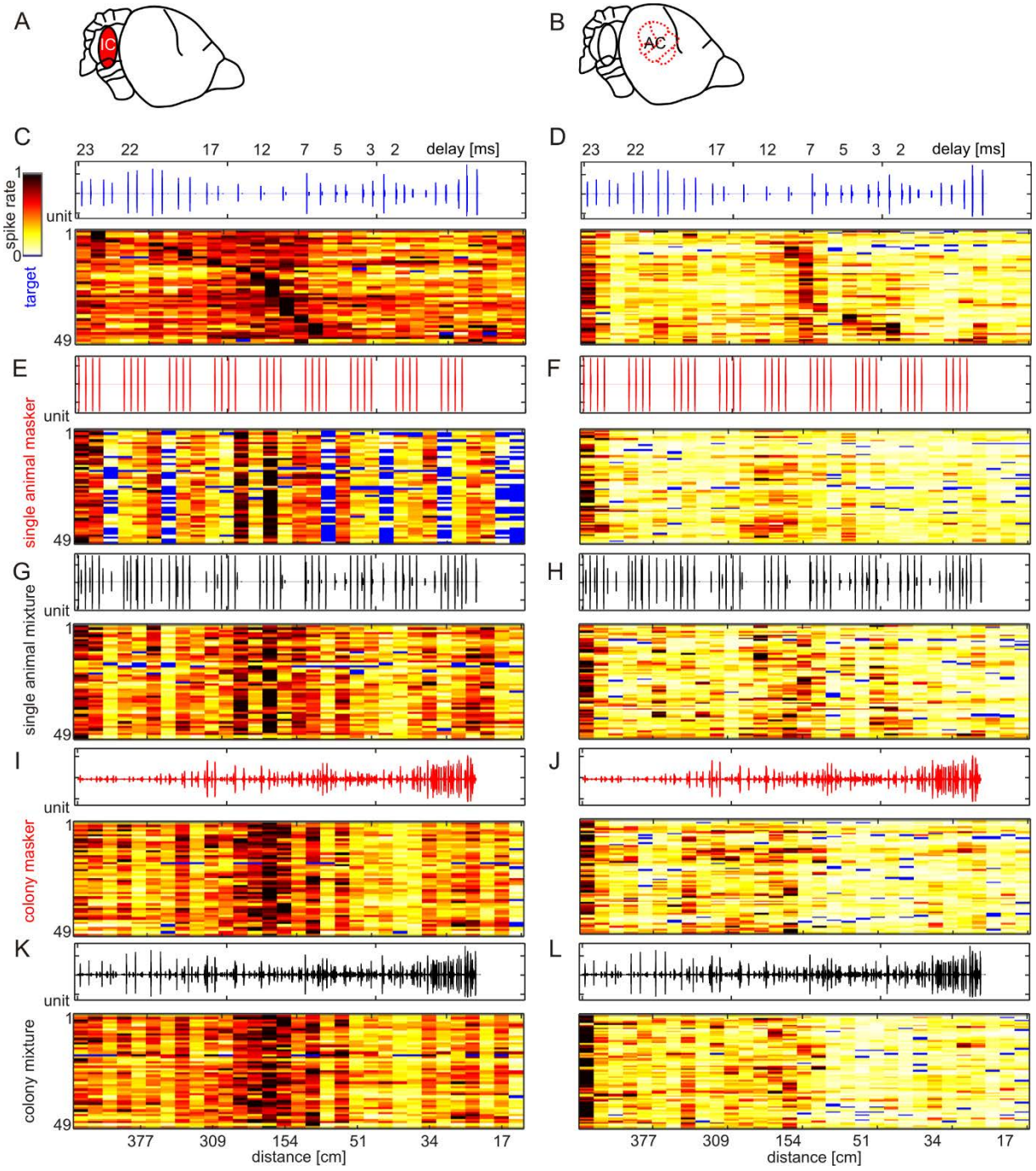


Figure S2 Neuronal responses of 49 collicular and 72 cortical units.
(A) Schematic lateral view on *C. perspicillata*'s brain. Recorded brain areas are highlighted (inferior colliculus (IC); auditory cortex (AC)). (B-F) Color-maps representing the neuronal activity (binsize according to duration of call-echo element) from 49 collicular (left column) and 72 cortical (right column) units in response to the target (B), single animal masker (C), single animal mixture (D), colony masker (E), and colony mixture (F) condition. Each row represents a particular unit and the units are ordered according to their best delay. Spike rate was normalized to the maximum bin for each unit and stimulus condition.

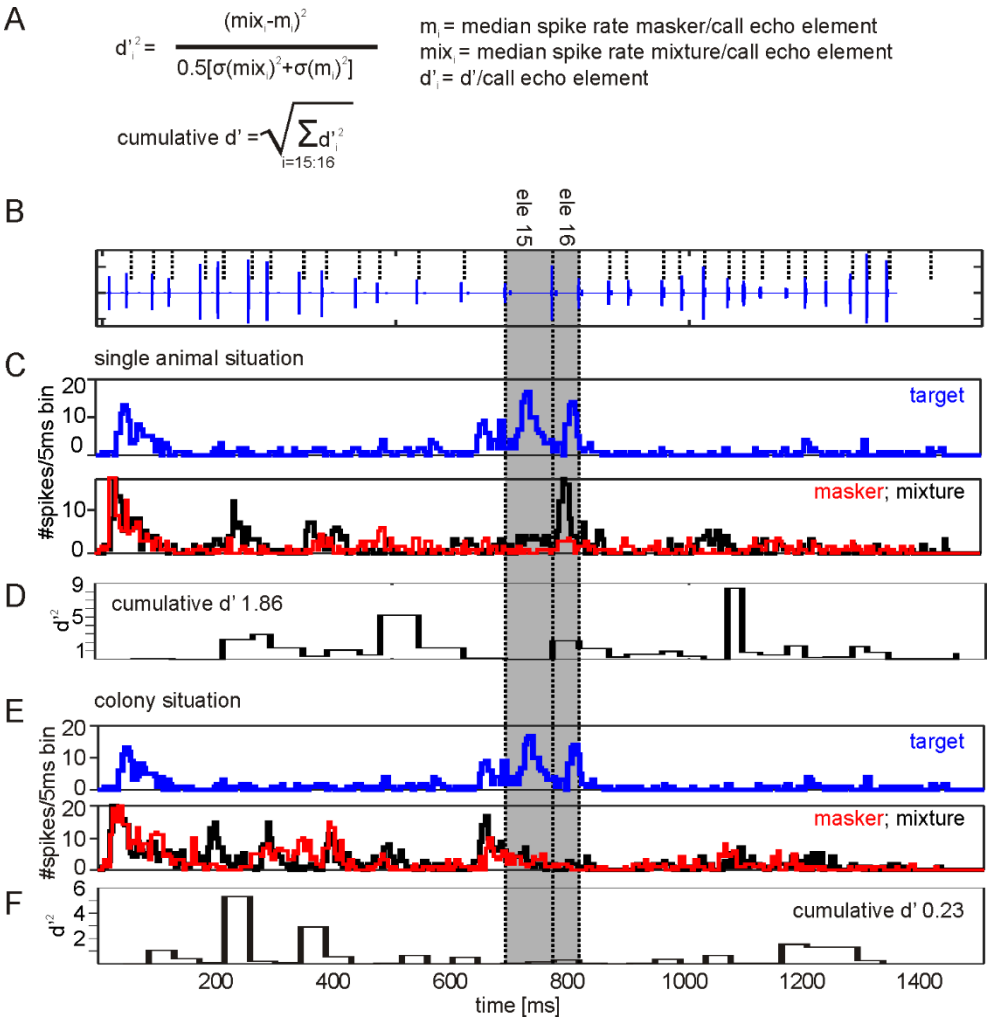


Figure S3 D' calculation exemplified by a cortical unit.
(A) Equations used to calculate a cumulative d' for each neuron. (B) Oscillogram of the target. Dashed vertical grey lines indicate the time borders of the response windows for each call-echo element. (C) PSTHs calculated in response to the target (blue), single animal masker (red) and single animal mixture (black). (D) Histogram shows the d'^2 for each call-echo element of the example unit. Note that high spike rate differences between the red and the black PSTH results in high d'^2 values. For calculating a cumulative d' for the unit, the d' values from call-echo elements to which the neuron responded to (ele 15 and ele 16) were summed. (E, F) The same figures as in (C, D) but in response to the colony masker and colony mixture. The response to the target was more preserved in the response pattern to the single animal mixture than to the colony mixture. The latter is evident when comparing the blue with the black PSTHs and it can be quantified by the cumulative d'.

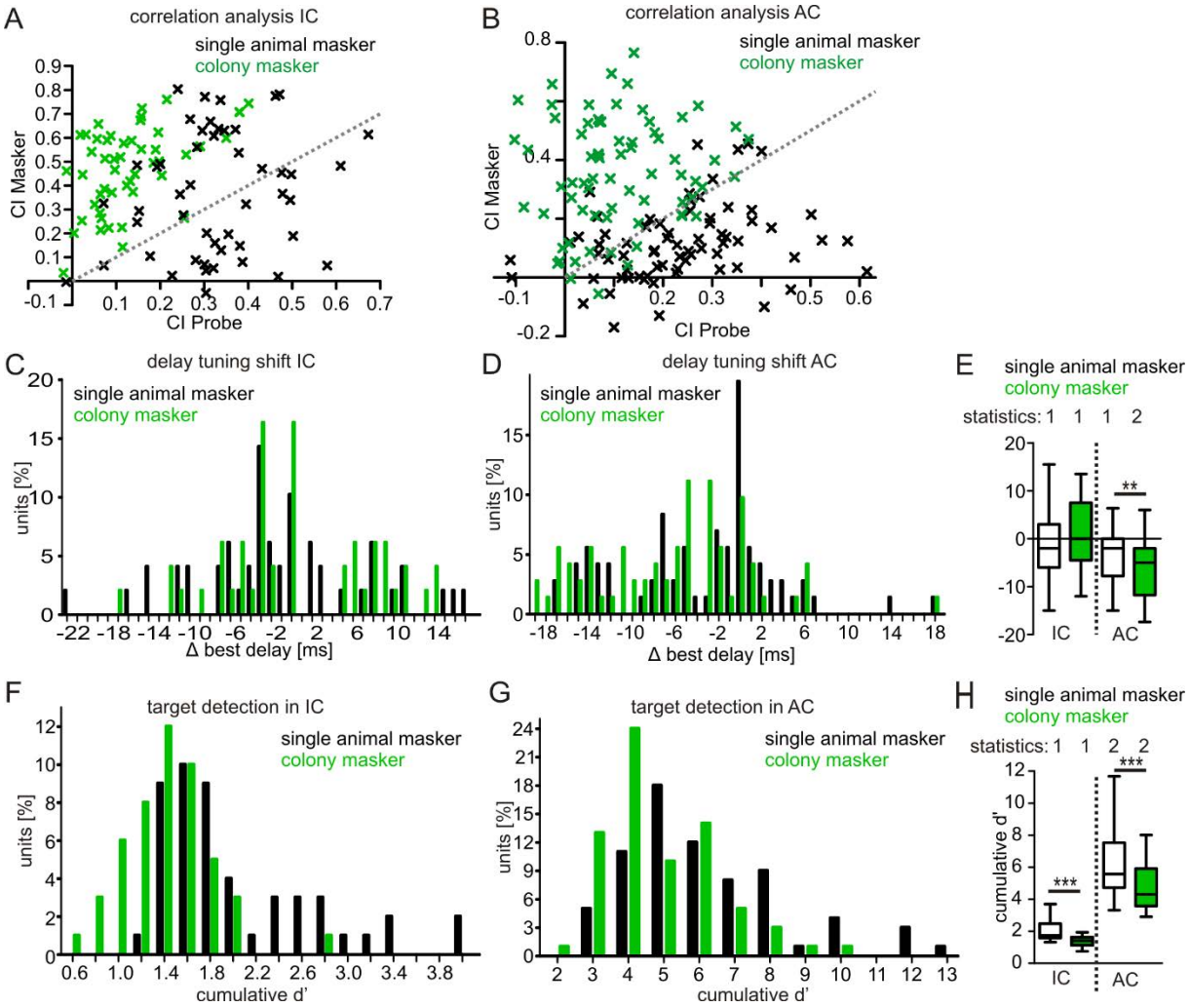
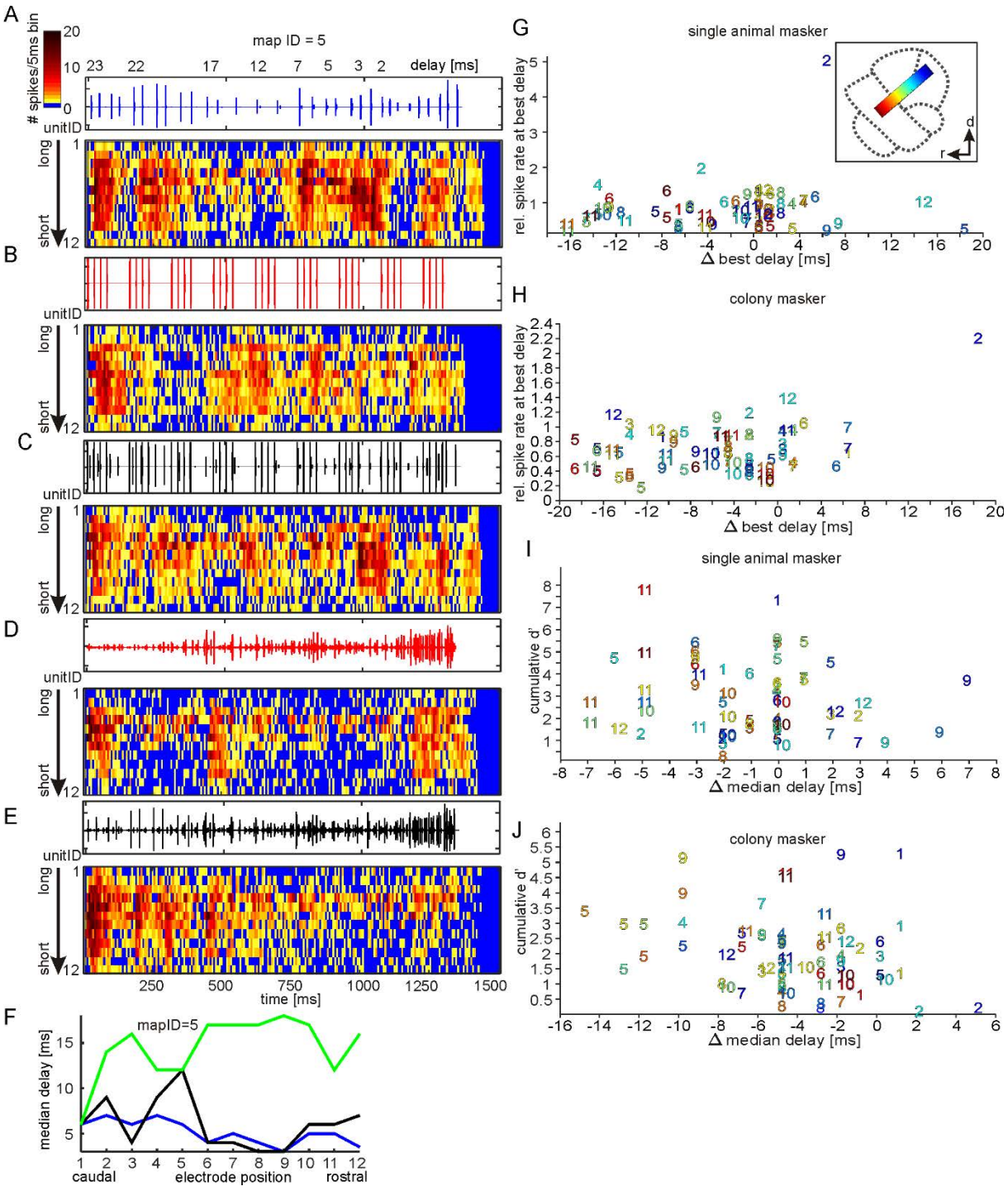


Figure S4 Robustness of neuronal processing in the presence of masker stimuli.
(A-B) Distribution of masker CIs are plotted against the target CIs from all collicular (A) and cortical (B) and units and both stimulus conditions. Values from single animal masker and mixture condition are plotted in black and the values from colony masker and mixture condition in green. (C, D) Histograms show the best delay shifts between target and mixture response in collicular (C) and cortical (D) units. Respectively, negative and positive values indicate delay shifts towards longer and shorter delays in response to the mixture condition. (E) Boxplots summarize the best delay shifts in the inferior colliculus (IC) and auditory cortex (AC). In the AC, the colony masker induced a higher best delay shift than the single animal masker. (F, G) Histograms show the target detection, represented by cumulative d' values, in the mixture situation in the IC (D) and AC (E). Note than in comparison to figure 4D and 4E all call-echo elements were considered to calculate the cumulative d' values. (F) Boxplots summarize the signal detection of the target in the presence of the masker. The cumulative d' and thus the neuronal signal detection were higher in the single animal than in the colony masker. Cumulative d' were also higher in the AC than in the IC.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10
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23 22 17 12 7 5 3 2 delay [ms]
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1
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250 500 750 1000 1250 1500
time [ms]
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mapID=5
median delay [ms]
15
10
5
1 2 3 4 5 6 7 8 9 10 11 12
caudal
electrode position
rostral
G
single animal masker
rel. spike rate at best delay
Δ best delay [ms]
d
r
H
colony masker
I
single animal masker
cumulative d'
Δ median delay [ms]
J
colony masker

Figure S5 Robustness of chronotopy in the presence of masker stimuli.
(A-E) Color-maps representing neuronal activity (binsize = 5 ms) from a cortical map in response to the target (A), single animal masker (B), single animal mixture (C), colony masker (D), and colony mixture (E) condition. Each row represents a unit. (F) Median delays in response to the target (blue), single animal mixture (black), and colony mixture (green) are plotted against the electrode positions for the example map. (G-H) Best delay shifts between the responses to the target and mixture were plotted against the relative spike rate to the best delay of the target sequence. Data points from single animal masker are plotted in (G) and from the colony masker in (H). In total twelve cortical maps were investigated and the map ID to which the particular data point belongs to is coded by a number (ranging from 1-12). Electrode position is color coded as represented in the scheme in (G). (I-J) Median delay shifts between the responses to the target and mixture were plotted against the cumulative d'. Only call-echo elements to which the neuron was sensitive to in the target sequence was considered to calculate the cumulative d'. Data points from single animal masker are plotted in (I) and from the colony masker in (J).

Discussion

1. How does the temporal pattern of the sequence influence the neuronal processing of echolocation signals?

1.1 Echolocation sequences induce suppression

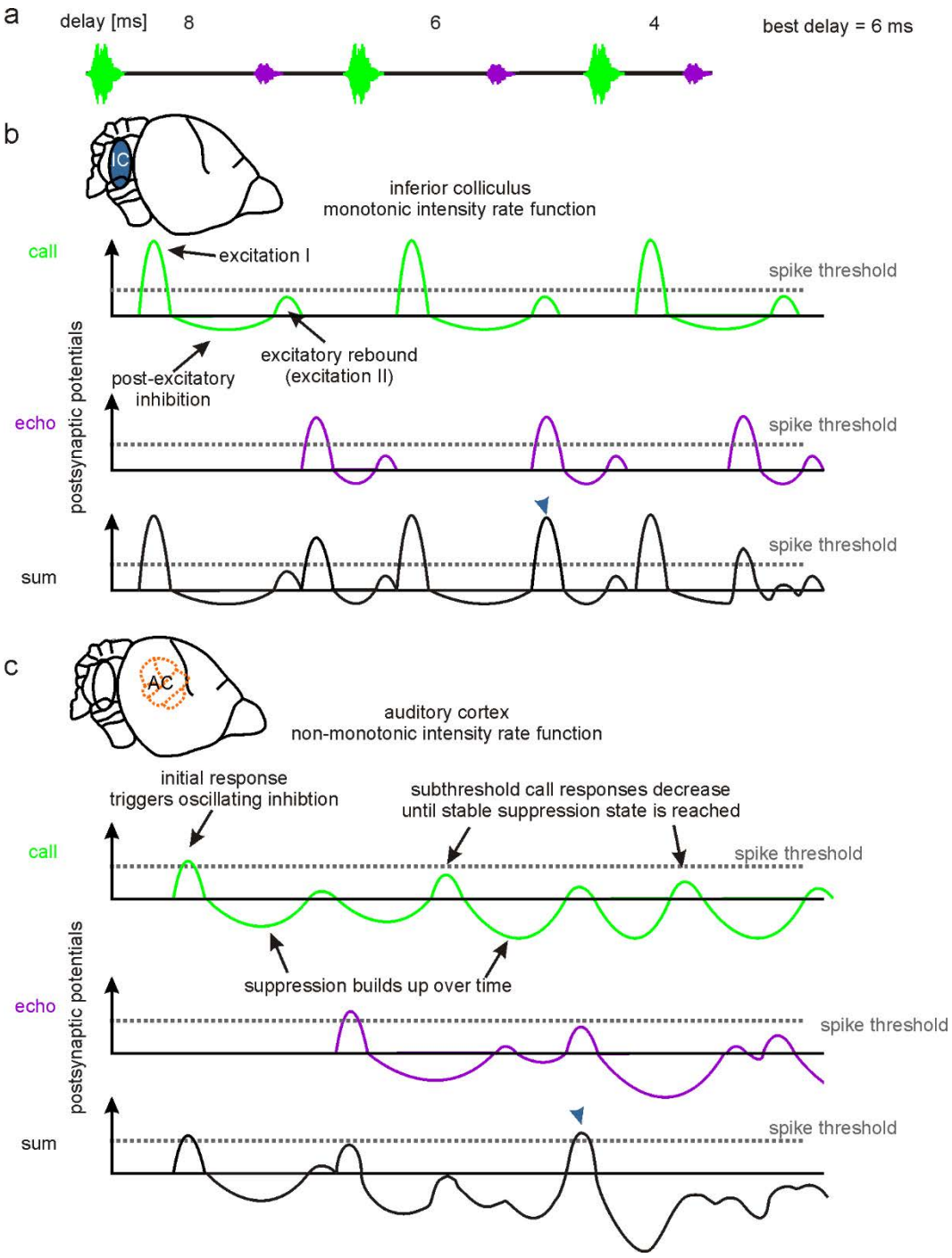
When stimulated with high acoustic rates, neurons usually do not reliably respond to each acoustic event, a phenomenon called neuronal suppression (for review see: Joris et al. 2004; Wang et al. 2008; Simmons and Simmons 2011). Neuronal suppression is interpreted to degrade temporal processing of repetitive stimuli. However, it remains unexplored whether neuronal suppression also degrades the temporal processing in animals that behaviorally rely on high stimulus rates. Echolocation sequences induce neuronal suppression in the AC and IC of *C. perspicillata* (Beetz et al. 2016b; Beetz et al. submitted A). In contrast to degrade temporal processing, suppression enhances the sharpness of temporal tuning to particular call echo elements of the sequence. This corresponds to results which show that neuronal tuning to durations (Zhou and Jen 2006), frequencies (Smalling et al. 2001; Jen et al. 2001), echo delays (Wong et al. 1992; O'Neill and Suga 1982), amplitude (Galazyuk et al. 2000), and azimuthal positions (Wu and Jen 1996) is more selective at high than at low stimulus rates.

In the IC and AC, suppression was particularly strong following high spike rates (Beetz et al. 2016b; Beetz et al. submitted A). Similar time courses of suppression indicate that the suppression underlies common mechanisms, in both brain regions. In nervous systems, excitation and inhibition are tightly coupled (Isaacson and Scanziani 2011; van Vreeswijk and Sompolinsky 1996; Wehr and Zador 2003; Keine et al. 2016). Inhibition follows excitation and is usually longer lasting than excitation (for review see: Wu et al. 2011; Isaacson and Scanziani 2011) which results in post-excitatory inhibition (Aitkin et al. 1966; Nelson and Erulkar 1963; Litovsky and Yin 1998). Post-excitatory inhibition has been demonstrated in the IC (Suga 1964; Covey et al. 1996) of FM-bats. Subthreshold excitations could also be followed by inhibition (Asari and Zador 2009) which explains why cortical suppression was detected without preceding spike activity, in bats (Edamatsu and Suga 1993; Beetz et al. 2016b). According to the current view, echolocation signals evoke inhibition followed by excitation (Figure 3b). However, the results of the present thesis leads to the hypothesis that echolocation signals evoke an excitation (excitation I) followed by inhibition (post-excitatory inhibition) and a rebound excitation (excitation II; Figure 5). The strengths of excitation and inhibition are proportionally related to each other. Thus, inhibition increases with excitation (Isaacson and Scanziani 2011).

Although cortical and collicular suppression could share common mechanisms in *C. perspicillata*, it is evident that suppressive effects are stronger in the AC than in the IC. Collicular suppression does not

degrade the tracking ability to the stimulus envelope as cortical suppression does (Beetz et al. 2016b). The former improves the signal-to-noise ratio of the neuronal response (Beetz et al. submitted A). On contrary, suppression vastly degrades neuronal tracking ability to the echolocation sequence, in the cortex (Beetz et al. 2016b). How could the relatively strong cortical suppression be explained? Cortical neurons receive vast inhibitory input. Inhibitory effects could arise intra- (Ojima 2011) and extracortically (Bayazitov et al. 2013). Intracortically, inhibition is generated by GABA (Fubara et al. 1996; Winer et al. 2011; Isaacson and Scanziani 2011). In bats, GABA-mediated intracortical inhibition sharpens frequency and delay-tuning curves of cortical neurons (Suga and Manabe 1982; Chen and Jen 2000; Hechavarría and Kössl 2014).

In contrast to bats, mechanisms of cortical suppression have been studied in rodents (Wehr and Zador 2005; Tan et al. 2004; Bayazitov et al. 2013). Cortical forward suppression is induced by two repetitive tones and it is composed of a short and a long lasting component (Wehr and Zador 2005). The short component lasts for 50-100 ms and it is intracortically mediated by $GABA_A$-receptors (Wehr and Zador 2005). The long component lasts for several hundreds of milliseconds and it is based on presynaptic thalamocortical synaptic depression (Bayazitov et al. 2013). How could the results in rodents explain the cortical suppression seen in bats? The strength of cortical suppression depends on stimulus history and increases during the stimulation (Beetz et al. 2016b). The long lasting suppressive effects could be mediated by oscillations between excitation and inhibition (Figure 5c). Oscillations of the membrane potential are common in the cortex (Isaacson and Scanziani 2011). Therefore, I propose that each excitation, including the rebound excitation, is followed by an inhibition and vice versa. When the bat is stimulated by a single biosonar signal, inhibition and excitation decays and the neuron's membrane potential goes back to its resting potential over time. However, when the bat is stimulated by an echolocation sequence, each acoustic signal of the sequence prolongs the oscillation and prevents the neuron to resettle to its resting membrane potential. In conclusion, cortical oscillations with its relatively long lasting inhibitory inputs reduce the neuron's membrane potential (Wu et al. 2011). Cortical neurons usually have a broader inhibitory than excitatory tuning curve (Isaacson and Scanziani 2011; Wu et al. 2011). Thus, non-preferred stimulus features evoke stronger inhibition than excitation. Corresponding to the bats, delay-tuned neurons are inhibited by non-best delays (Hechavarría and Kössl 2014). The inhibition in response to non-preferred delays and the cortical oscillations decrease the membrane potential over time (black graph in Figure 5c). Only strong excitatory inputs pass the increased spike threshold of the neurons and elicit spikes in delay-tuned neurons, a phenomenon also called the "iceberg effect" (Isaacson and Scanziani 2011). A spike threshold increase in response to a high stimulus rate has also been described in cats (Phillips et al. 1989), bats (Chen and Jen 1994; Jen and Chen 1998), and gerbils (Donaldson and Rubel 1990).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8
6
4
best delay = 6 ms
b
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inferior colliculus
monotonic intensity rate function
call
excitation I
spike threshold
post-excitatory inhibition
excitatory rebound (excitation II)
postsynaptic potentials
echo
spike threshold
sum
spike threshold
c
AC
auditory cortex
non-monotonic intensity rate function
initial response triggers oscillating inhibtion
subthreshold call responses decrease until stable suppression state is reached
call
spike threshold
suppression builds up over time
postsynaptic potentials
echo
spike threshold
sum
spike threshold

Figure 5 Hypothetical mechanism of processing acoustic sequences. (a) Oscillogram of an exemplary echolocation sequence containing three call echo pairs with echo delays of 8, 6, and 4 ms. Calls (green) are more intense than echoes (violet). (b) Postsynaptic potentials (PSPs) arriving at a delay-tuned collicular neuron with a best delay of 6 ms. Collicular neurons often show a monotonic intensity-rate function. Thus, the neuron more strongly responds to the calls (green curve) than to the echoes (violet curve). When stimulating with an echolocation sequence, the collicular neuron fires reliably in response to each acoustic event. Each suprathreshold excitatory input (excitation I) is followed by a post-excitatory inhibition and a subthreshold excitatory rebound. The duration of the inhibition depends on the strength of the preceding excitation I. When the PSPs in response to the calls and echoes are summed (black curve), then the collicular neuron still respond to each acoustic event. However, the strongest echo response occurs at the call echo element with the best delay of the neuron (blue arrowhead). (c) Cortical PSPs in response to the sequence considering only the calls (green curve) and the echoes (violet curve). The best delay of the cortical neuron is 6 ms. In comparison to the collicular neuron, the cortical neuron only respond to the first acoustic event of the sequence (initial response). Faint echoes lead to stronger excitatory inputs than intense calls, because of the neuron's non-monotonic intensity rate function. Note that even excitation II is followed by an inhibition, thus the cortex oscillates between excitatory and inhibitory inputs during the stimulation. The initial response triggers the oscillation onset and the oscillation maintains the cortex in a suppressed state. Synaptic inputs from subsequent acoustic events are added. Thus, the suppression builds up over time until reaching a stable state. In response to the call echo sequence (a) the cortical neuron shows an initial response to the call and recovers from suppression only, if excitation II elicited by a call coincides with excitation I evoked by an echo (blue arrowhead). Note that the strongest suppression is reached immediately after the strongest excitation.

2. How do neurons process echolocation sequences that contain echo information from many different objects?

2.1 Cortical neurons most selectively process information from the nearest object and collicular neurons can process information from each object.

When orientating in complex environments, bats spatially focus their sonar beam on single objects (Surlykke et al. 2009a; Surlykke et al. 2009b) and they often shift their sonar beam in a saccadic way between objects (Seibert et al. 2013; Fujioka et al. 2014). These adaptations reduce the risk of generating multiple echoes that follow a call. However, the spatial restriction of the sonar beam is limited and highly variable (Linnenschmidt and Wiegrebe 2016). Thus, bats have to be able to cope with echolocation sequences where multiple echoes follow a call.

According to the previously described neuronal suppression, one might expect that the enhanced acoustic rate in the multi-object sequence increase the suppressive effects (Beetz et al. 2016b). Cortical data from the present thesis confirm this assumption (Beetz et al. 2016a). Cortical neurons predominantly respond to the first echo following the call. Subsequent echoes usually evoke weak or no response. The cortical response to the multi-object sequence can be explained with the suppression mechanisms introduced in the previous section. The excitatory response (excitation I) to the first echo is followed by a long lasting inhibitory input that prevents the cortical neurons to spike in response to the subsequent echo.

Because of the aforementioned cortical oscillations, the suppressive effects are longer lasting in the AC than in the IC.

The cortical results from anaesthetized and passively listening bats cannot explain the behavioral data which show that bats can attend their sonar beam towards distant objects and may suppress the processing of echo information from the nearest object (Fujioka et al. 2016; Amichai and Yovel 2017). Although the influence of the animal's attention on cortical processing could not be tested in the present thesis, the neuronal data from the IC could explain the behavioral data. When stimulated with the multi-object sequence, the collicular neurons responded to each echo (Figure 5b; Beetz et al. submitted A). The response pattern of the collicular neurons could be explained by the lack of a long lasting suppressive effect in the IC. In contrast to the cortex, oscillations of excitation and inhibition seem to be weak or absent in the IC. Although, the collicular neurons most strongly respond to echoes of the nearest object, the response to subsequent echoes is less suppressed in the IC than in the AC. In summary, the IC could process spatial information from each object, whereas the AC most selectively processes echo information from the nearest object.

3. How do the neuronal responses to single- and multi-object sequences change the view on the mechanisms of delay tuning?

3.1 Call echo discrimination

The neuronal responses to the multi-object sequence indicate that call echo discrimination is not based on intensity differences between the call and echo. It is believed that the most intense signal of a call echo element is defined as the call and the faint one as the echo. If this strategy underlies call echo discrimination, then the first echo of the multi-object sequence could be misassigned to be the corresponding call for the subsequent echo. Instead of processing the delay between the correct call and the echo, the neurons would process the delay between two consecutive echoes. According to the results of the present thesis, a call echo discrimination based on intensity can be excluded (Beetz et al. 2016a). Calls and echoes were never misassigned by the neurons because the neurons were immediately suppressed after the first echo.

Call echo discrimination could be simply based on the computation of the time points of the call and echo. Behavioral results from CF-FM bats showed that a sensitive time window is opened after call emission (Roverud and Grinnell 1985b; Neuweiler 1990). Any subsequent biosonar signal is interpreted as the echo and closes the time window. If no echolocation signal is perceived, then the time window closes after a particular time (Neuweiler 1990). If call echo discrimination occurs based on a time window, then the window should be respectively opened and closed by the call and echo. The time window would also

explain why a call echo misassignment has never occurred when stimulating the bat with the multi-object sequence (Beetz et al. 2016a).

3.2 Delay tuning mechanism

The neuronal pattern in response to the echolocation sequences gives us new insights into the delay tuning mechanisms. First, I want to summarize the basic response properties that underlie my following hypothesis on delay tuning: i) Delay tuning is based on a coincidence detection of two excitatory inputs, one elicited by a call and the second one by the echo. ii) Each echolocation signal induces three changes in the membrane potential of delay-tuned neurons. The neurons receive an excitatory input (excitation I) that is followed by an inhibition and an excitatory rebound (excitation II). iii) Long lasting suppressive effects at cortical level could account for the different neuronal response properties between the IC and AC.

Collicular neurons spike in response to each call and echo of the echolocation sequence (black graph in Figure 5b). The response to the call echo element encoding the best delay of the neuron is facilitated (blue arrowhead in Figure 5b), because excitation II in response to the call (green graph in Figure 5b) coincide with excitation I in response to the echo (violet graph in Figure 5b). In cortical neurons, the same mechanism could be implemented, except that the cortical oscillation decreases the neuron's membrane potential which transfers the suprathreshold excitations I into subthreshold excitations I (Figure 5c). According to the here proposed mechanism, the response to the call need not to be delayed from the response to the echo. The latter explains that delay tuning can be characterized with equally intense calls and echoes. The mechanism of delay tuning which has been described in the literature cannot explain that neurons show delay tuning to equally intense calls and echoes (Figure 3).

According to the hypothetical mechanism of delay tuning, the neuron's best delay depends on the duration of inhibition and the neuron's latency. Although not explicitly quantified, an increase of the stimulus intensity shifts the delay tuning towards longer delays, in some neurons (Figure 6a). Almost all delay-tuned neurons have a non-monotonic intensity rate function (Hechavarría and Kössl 2014). In other words, the sensitivity and response strength decrease with increasing sound levels. Non-monotonicity is already created in the IC by the influence of GABA (Yang et al. 1992; Park and Pollak 1994, 1993). But, the relative amount of non-monotonic neurons increases from subcortical to cortical areas (cat: Barone et al. 1996; bat: Suga and Manabe 1982). According to the non-monotonicity, the duration of inhibition is elongated with increasing intensity. Therefore, the excitatory rebound (excitation II) gets delayed with increasing intensity and thus the best delay is also shifted towards longer delays (Figure 6b).

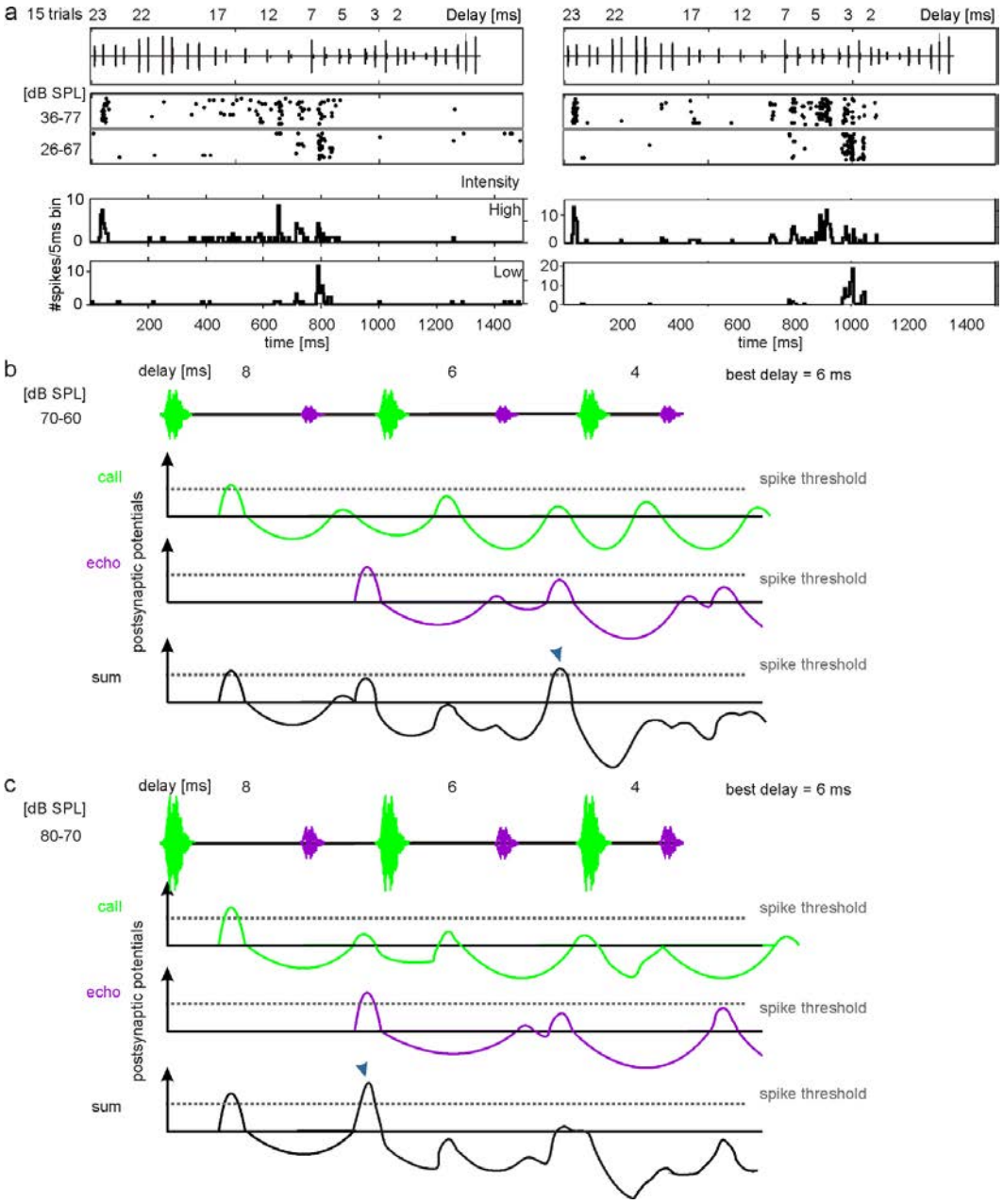

Figure 6 Intensity dependent best delay shifts in the AC. (a) Neuronal activity from two representative cortical neurons in response to an echolocation sequence. (Top) Oscillogram of the sequence with some indicated echo delays. The sequence was presented to the bat in two different intensity ranges. High intensity ranged between 36 and 77 dB SPL and low intensity between 26-67 dB SPL. Neuronal responses are shown as raster plots (middle) and as post-stimulus time histograms with a binsize of 5 ms (bottom). Note, that the neuron's best delays shifts towards longer delays, indicated by earlier responses, with increasing signal intensity. (b, c) Hypothetical mechanism that explains the shift of the best delay. The subfigures are organized as in figure 5. (b) When stimulated with a sequence containing signals at intensities of 70 and 60 dB SPL, a cortical neuron partially recovers from suppression at the call echo element that encodes a delay of 6 ms (blue arrowhead). (c) By increasing the stimulus intensity, the recovery from suppression is shifted towards the call echo element encoding a delay of 8 ms (blue arrowhead). Note, that the tip of the excitation representing the recovery is narrower in (c) than in (b). Thus, the relative position between the excitatory rebound (green curve) and the excitation (violet curve) could affect the response duration at the point of partial recovery.

4. How do acoustically interfering sounds influence echolocation behavior and neuronal processing of echolocation sequences?

4.1 In noisy environments, *C. perspicillata* increases its sensory acquisition rate which could correct erroneously processed distance information

Bats often fly in groups resulting in a multitude of high frequency sounds that could act as maskers (Parsons et al. 2003; Levin et al. 2013). Under these conditions, echolocation is challenging because every bat has to identify its own echolocation signals. Although actively avoiding noisy environments (Beetz et al. submitted B; von Frenckell and Barclay 1987), bats cannot fully prevent flying under noisy conditions. Therefore, they show a certain repertoire of behavioral adaptations that allow them to orientate in noisy environments. For simplicity, these adaptations are referred to as jamming avoidance responses (JAR). Before discussing the JARs that *C. perspicillata* showed in the present thesis, I want to review the JARs that have been documented in other bat species.

The term JAR initially comes from studies on weakly electric fish (Watanabe and Takeda 1963; Bullock et al. 1972b; Bullock et al. 1972a). In electric fish, the JAR was exclusively defined as spectral shifts of the fish's electric signals to reduce the overlap with the conspecific's signals. The JARs reported in bats can be subdivided into three categories. i) Individual-specific calls: Each bat has an innate echolocation call design that is distinguishable from calls of conspecifics (Amichai et al. 2015; Masters et al. 1991; Obrist 1995; Jones et al. 1991; Schnitzler et al. 1987; Brigham et al. 1989; Masters et al. 1995). ii) Changes at the sensory level: During complex motion tracking, bats increase head waggles which might improve the spatial localization of the echo source (Wohlgemuth et al. 2016a). The directionality of hearing can further be increased by adjusting the pinna's shape (Gao et al. 2011). By increasing the inter-pinna separation, the sensitivity to echoes coming from the periphery increases (Wohlgemuth et al. 2016a). iii) Actively adjusting the call design and emission pattern: By changing different call parameters or the emission pattern, bats could make their echolocation signals distinct from the ones of conspecifics (Habersetzer 1981; Miller and Degn 1981; Ratcliffe et al. 2004; Ibanez et al. 2004; Ulanovsky et al. 2004; Cvikel et al. 2015; Gillam and McCracken 2007; Takahashi et al. 2014; Tressler and Smotherman 2009; Hage et al. 2013; Gillam et al. 2007). Bats could change spectral parameters like the call bandwidth and/or peak frequency. The peak frequency is the frequency that contains the maximum energy of the call. The bats could also increase the signal-to-noise ratio by increasing call intensity, an adaptation usually referred as the "Lombard effect" (Amichai et al. 2015; Hage et al. 2013; Takahashi et al. 2014; Tressler and Smotherman 2009; Luo et al. 2015; Simmons et al. 1978). In addition, some bats increase the call duration (Amichai et al. 2015). *Eptesicus fuscus* adds a short CF component at the end of each FM sound. This component marks the call and echo which facilitates call echo detection (Simmons et al. 1979; Simmons

et al. 1975; Tressler and Smotherman 2009). Instead of changing certain call parameters, bats could also adjust their call emission pattern to decrease the risk of being jammed. Some bat species alternate between two call designs that differ in the call spectrum (Obrist 1995; Roverud and Grinnell 1985a, b). This adaptation allows a higher call rate by emitting two calls before receiving any echo (Behr and von Helversen 2004; Jung et al. 2007). The arising echoes differ in their frequency spectra which makes their discrimination feasible (Hiryu et al. 2010). Other bats reduce their call rate (Adams et al. 2017) and temporally even cease to emit calls (Jarvis et al. 2013). It has been proposed that bats might eavesdrop on echolocation signals from conspecifics and can use the signals for orientation (Barclay 1982; Leonard and Fenton 1984; Balcombe and Fenton 1988). Interestingly, behavioral results support the hypothesis because lagging bats stop call emission when they share flight paths with a leading bat (Chiu et al. 2008). According to a modeling study, a call rate decrease is only beneficial for orientation, if the bats eavesdrop on each other (Lin and Abaid 2015). Some bats also increase their call rate in response to acoustic noise (Roverud and Grinnell 1985a). Although an increase of the call rate might increase the acoustic interference, it also lowers the chance of obstacle collisions (Lin and Abaid 2015). Lastly, bats increase their rate of grouping calls when orientating in noisy environments (Roverud and Grinnell 1985a; Luo et al. 2015; Beetz et al. submitted B). Grouping the calls can differently facilitate echolocation performance. First, a known periodicity of echo arrivals allows echo identification based on prediction (Wohlgemuth et al. 2016a; Petrites et al. 2009; Wheeler et al. 2016; Suga et al. 1983). Second, corresponding to the call intervals (Beetz et al. submitted B) and the bat's flight speed (Thies et al. 1998), a call group containing three calls (triplet) encodes similar echo delays in the range of ± 1ms. Thus, the information loss that the bat has if one of the call echo pairs gets masked is minimal and the bat can rely on non-masked call echo pairs. In conclusion, bats may have different behavioral strategies to reduce the risk of acoustic interference and jamming. Not only each species (Ulanovsky et al. 2004) but also each individual may use different strategies (Tressler and Smotherman 2009). Thus, bats may have a toolkit of different combinable JARs (Hage et al. 2013; Luo et al. 2015).

Despite of the large amount of behavioral data from bats, it remains unknown whether the JARs actually improve the signal detection at high noise levels. The JAR shown by *C. perspicillata* results into an increased acoustic rate (Beetz et al. submitted B). With respect to the previously described neuronal suppression, I wonder whether the neurons can actually process relevant echolocation information under the increased stimulus rates generated by the masker and the bat's JAR. For neurophysiological experiments, two different noisy environments were simulated (Beetz et al. submitted B). One scenario mimicked the stimulus situation that a bat perceives when encountering a conspecific (single animal masker) and the second one when flying in a colony (colony masker). Data from the IC revealed that collicular neurons might profit from the JAR in both stimulus scenarios, because the relatively weak collicular suppression does not render the IC into a completely suppressed state. In cortical neurons,

processing of echolocation information was more strongly affected by the colony than by the conspecific masker. However, when considering the neuronal response of simultaneously recorded cortical neurons then the signal detection ability in the presence of the maskers is highly preserved (Beetz et al. submitted B). In conclusion, the JAR shown by *C. perspicillata* should facilitate echolocation performance by guaranteeing signal detection ability of the neurons. The high stimulus rate induced by the masker did not render the cortex into a silent mode. Echolocation signals evoking strong excitatory input to delay tuned neurons can still elicit spikes in cortical neurons which are in a suppressive state (Beetz et al. 2016b; Beetz et al. submitted B).

5. How is the topographic representation of echo delays along the cortex affected by stimulation with natural echolocation sequences?

5.1 Chronotopic maps are less blurry when stimulating the bat with echolocation sequences

Topographic maps are evolutionarily fundamental and have been characterized in invertebrates (Beetz et al. 2015) and vertebrates (Suga 1965; Kaas 1997). In general, two different types of topographic maps are distinguished, structural and computational ones (Kaas 1997; Kössl et al. 2014). Structural maps are usually created at the sensory epithelium where receptors are topographically aligned according to a specific stimulus parameter. For instance, tonotopy represents a structural map. The frequency is represented in form of a place code in the cochlea (von Békésy 1960) and this tonotopic organization is maintained up to the AC (Larionow 1899). In contrast, computational maps are created by properties of the neuronal circuits. They represent an aspect of the sensory environment that is not mapped at the sensory surface (Kaas 1997; Knudsen et al. 1987; Kössl et al. 2014). The chronotopic map of bats represents a computational map.

Most topographic maps are blurry, variable, and they do not continuously represent a stimulus quality (Kaas 1997; Schreiner and Winer 2007; Hechavarría et al. 2013; Kaschube 2014). The degree of blurriness usually increases along the ascending sensory pathway with maximal blurriness at secondary cortical fields (Kaas 1997). The blurriness can be based on different aspects. i) High inter-individual differences make it problematic to derive a map by pooling data from many animals (Bandyopadhyay et al. 2010; Fitzpatrick et al. 1998b; Guo et al. 2012; Merzenich and Brugge 1973; Merzenich et al. 1973, 1975) ii) Different stimulus parameters used to characterize a map (Hechavarría et al. 2013; Yokota et al. 2012) make mapping experiments quite subjective. iii) Overlapping receptive fields from adjacent neurons make the map less continuous (Hechavarría et al. 2013; Beetz et al. 2015; Knudsen et al. 1987); iv) Neurons, especially at the cortex level, often receive multiparametric inputs. Thus, neurons encode different stimulus features like spectral, temporal, and intensity (O'Neill and Suga 1979; Suga 1994; Suga

et al. 1978; Schreiner and Winer 2007). v) Neuronal responses are often context dependent (Suga 1994). The animal's attention, motivation, and experience can induce receptive field plasticity (Osmanski and Wang 2015; Sarter et al. 2005; Everitt and Robbins 1997). vi) At cortical level, neurons with similar receptive fields are organized in modular columns (Mountcastle 1997; Jones 2000; Suga and Manabe 1982; Fitzpatrick et al. 1998a). However, the receptive fields within a column can differ along the depth, thus creating a columnar heterogeneity (Yokota et al. 2012; Atencio et al. 2009). vii) Spatial and temporal scales of investigation (Liberti et al. 2016; Bandyopadhyay et al. 2010): According to global signals, like local field potentials or multiunit data, the response pattern is stable leading to globally homogenous map. But, at single cellular level the response pattern can be quite plastic which results into a locally heterogeneous map. Receptive field plasticity could additionally change topographic maps over the day (Schreiner and Winer 2007). viii) Neuronal connections between neurons with different receptive fields (Chklovskii and Koulakov 2004).

Single electrode recordings revealed that the chronotopic map of *C. perspicillata* is blurry (Hechavarría et al. 2013; Hagemann et al. 2010). With the results of the present thesis I could test whether the blurry chronotopic map was man-made by using artificial stimuli or by pooling recordings from different time points. Alternatively, the blurriness could be real and relevant for neuronal processing (Hechavarría et al. 2013; Kaschube 2014). The blurriness was preserved with simultaneous recordings from different spots of the map which reveals that the blurriness is not based on pooling neurons recorded at different time points (see map in response to "element situation" in Beetz et al. 2016b). However, the blurry map was rendered into a smooth one when using echolocation sequences instead of single call echo elements as stimuli (see map in response to "sequence situation" in Beetz et al. 2016b). The chronotopic map is even robust against acoustic maskers (Beetz et al. submitted B). Thus, sequence induced cortical suppression has a strong influence on shaping and maintaining a smooth topographic map. The chronotopic map represents a nice example in which blurriness mostly originates from using artificial and behaviorally irrelevant stimulus settings, instead of representing a real aspect of a topographic map. Therefore, future studies should carefully select the stimuli when characterizing receptive fields and topographic organization of neurons (Schreiner and Winer 2007).

So far, all investigated bats, except *E. fusucs* and *Myotis lucifigus* have a chronotopic map at the level of the cortex (Kössl et al. 2014). The map robustness against interfering maskers (Beetz et al. submitted B) and its occurrence in many bat species might indicate that the maps indeed have a functional role for the bats. Nonetheless, whether topographic maps have a functional role is one of the most controversially discussed topics in neuroscience (Kaas 1997; Weinberg 1997). Most scientists agree that the formation of topographic maps is energetically efficient to minimize connections and redundancy within a neuronal circuit (Chklovskii and Koulakov 2004; Weinberg 1997; Kössl et al. 2014). The vicinity of neurons with similar receptive fields may support circuit plasticity (Schreiner and Winer 2007). During

learning, neuronal circuits are modified which is reflected by changes in the neuronal receptive fields (Ma and Suga 2003; Weinberger 1993, 1998; Fritz et al. 2005; Buonomano and Merzenich 1998). The receptive field changes are usually subtle and smooth which preserves the topographic organization. For instance, a cortical neuron with a best delay of 2 ms can change its best delay in a range of about 8 ms, but not to 20 ms (Suga and Ma 2003). A topographic organization allows rapid receptive field shifts by changing synaptic weights and the balance between excitation and inhibition (Schreiner and Winer 2007). According to the receptive field plasticity, stimulus features that rapidly change depending on the behavioral context should be topographically organized. The environments, in which bats orientate, can suddenly change from open space to cluttered surroundings. When flying in cluttered environments, the bats mostly rely on neurons tuned to short distances. On contrary, long delay-tuned neurons are more required when orientating in open space. Results from the bat species *P. discolor* demonstrate, that depending on the environmental context, the proportion of short and long delay representation is dynamically modified in the cortex (Bartenstein et al. 2014).

E. fuscus and *M. lucifugus* orientate primarily in open space and hunt for insects in uncluttered environments (Kössl et al. 2014). Thus, receptive field plasticity, induced by sudden habitat changes during hunting are not as relevant as for bats hunting or flying in cluttered environments. Besides, the lack of evidence does not exclude the existence of a map in these species. Mapping experiments that use inappropriate stimuli may miss the topographic maps (Schreiner and Winer 2007; Beetz et al. 2016b).

How does the bat “see” the world under suppression?

The main purpose of the thesis was to test how neurons process natural echolocation sequences. According to the results, it is possible to imagine how echolocation signals may be processed under different behavioral conditions. When orientating in open space, each echolocation call could be reflected by one object only. The temporally precise and non-adapting neuronal responses of the IC could provide the bat with a coarse overview of the surrounding. High temporal precision of neuronal responses may be important to elicit rapid and well controlled motor responses. Note that, *C. perspicillata* usually uses call rates lower than 50 Hz. On contrary, collicular neurons of insectivorous bats that have to deal with call rates up to 200 Hz may have problems to synchronize their discharges with the echolocation sequence (Suga 1964b). Because of the wide range of call repetition rates, it is not surprising that rate selective neurons can be found in the IC (Sanderson and Simmons 2005) and AC (Suga et al. 1983; O'Neill and Suga 1979, 1982) of insectivorous bats. Some of these neurons can synchronize their discharges to call rates higher than 200 Hz. Cortical neurons of *C. perspicillata* respond selectively to particular call echo elements that encode specific distance information. The highly selective response may allow the bat to compute distance information in more detail which may give the bat a higher spatial resolution than in the

IC (Figure 7a). Pharmacological inactivation of the FM-FM area from the CF-FM bat *P. parnellii* supports this hypothesis (Riquimaroux et al. 1991). The cortical FM-FM area is crucial to distinguish between small distances because an inactivation with muscimol disrupted the bat's ability to discriminate echolocation sequences (repetition rate = 40 Hz) with small echo delay differences (Riquimaroux et al. 1991). However, coarse distance discriminations with echo delay differences of 36 ms could still be differentiated with an inactive cortex. Also, bats with an ablated cortex can echolocate (Suga 1969b) while IC ablated bats are incapable to echolocate (Suga 1969a).

When the bat enters a cluttered environment that is composed of many reflective objects, then each echolocation call may be followed by many echoes. Multiple echoes following a call are differently processed in the IC than in the AC. Thus, one could propose that both brain areas have also different functions for echolocation in cluttered environments. In the IC, each object specific echo can be processed. At cortical level, echoes from the nearest object are analyzed which allows a detailed analysis of one particular object (Figure 7b). Top-down influences like attention could shift the preference of neuronal processing to distant objects.

When the bat approaches its roost during homing then the chances of meeting conspecifics are relatively high. Echolocation signals of conspecifics acoustically interfere resulting into a noisy environment (Figure 7c). Behavioral results of the present thesis suggest that *C. perspicillata* temporally increases its sensory acquisition rate to reduce disorientation induced by maskers. Corresponding to the behavioral adaptation, the bat could overcome the information loss of the masked call echo element by grouping calls into functional units where each call echo element encodes a similar delay. Grouping the calls makes some of them dispensable because the bat only need echo information from one call out of the functional unit (Figure 7c bottom).

In the IC, the increased signal rate does not negatively affect neuronal processing because of the relatively weak collicular suppression. In the AC, the increased sensory acquisition rate may not be successfully processed by each neuron. However, at the population level wrong echolocation information coming from acoustic interferences can be corrected with the next call group emission. Thus, increasing the sensory update rate may help the bat to deal with moderate acoustic interferences. In extremely noisy environments, like in the roost an increased sensory update rate will not be sufficient to avoid jamming. But, under these conditions, the bat could rely more on its spatial memory.

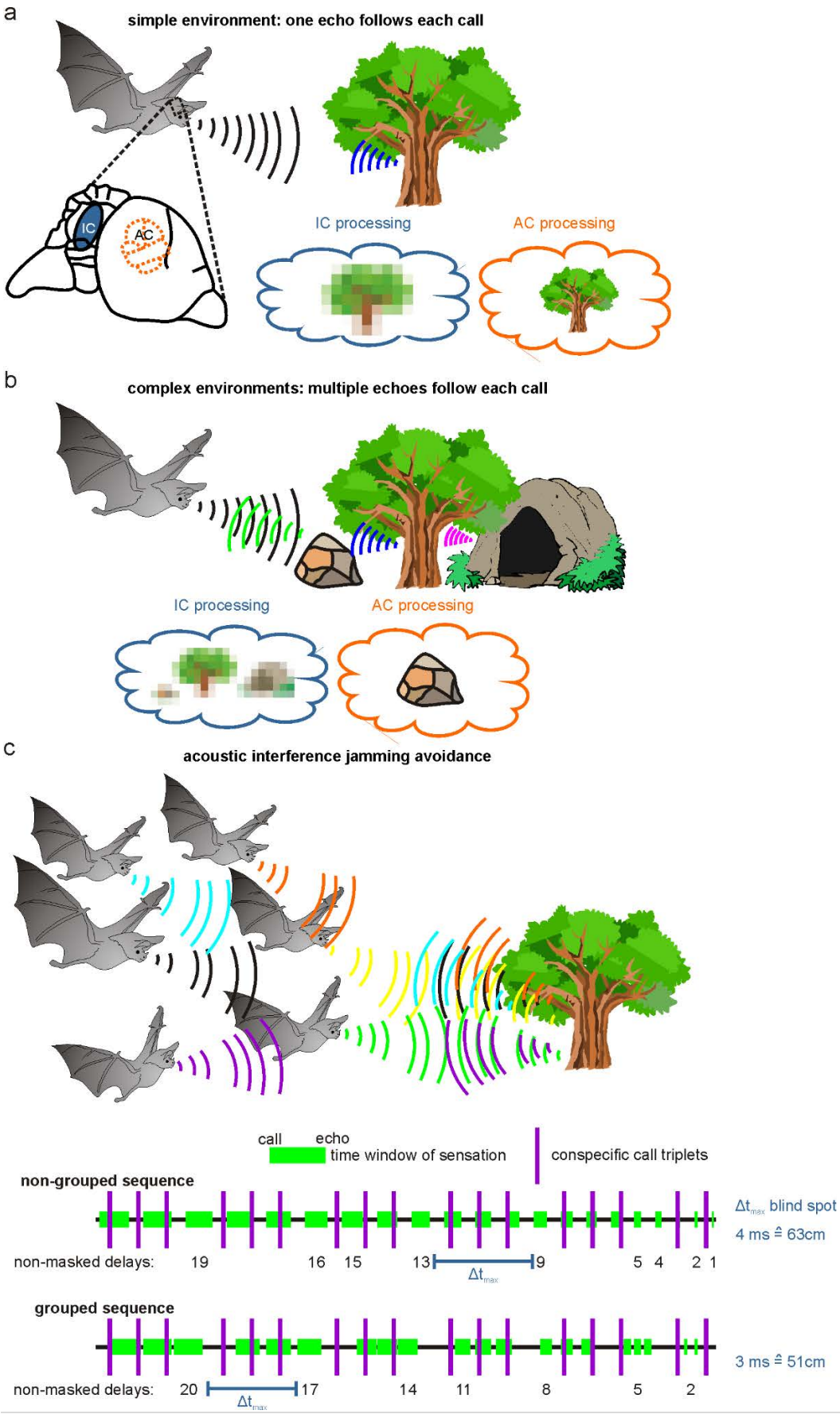
a
simple environment: one echo follows each call
IC
AC
IC processing
AC processing
b
complex environments: multiple echoes follow each call
IC processing
AC processing
c
acoustic interference jamming avoidance
call
echo
time window of sensation
conspecific call triplets
non-grouped sequence
Δt_max blind spot
4 ms ≙ 63cm
non-masked delays: 19 16 15 13 9 5 4 2 1
Δt_max
grouped sequence
3 ms ≙ 51cm
non-masked delays: 20 17 14 11 8 5 2
Δt_max

Figure 7 Summary showing a simplified version on how naturalistic echolocation sequences might be processed in the inferior colliculus (IC) and auditory cortex (AC). (a) In a simple environment with one prominent object, each echolocation call gets reflected once. IC neurons are broadly tuned to each acoustic event of the echolocation sequence. Delay tuning is relatively broad in comparison to cortical neurons. Thus, cortical neurons allow a higher spatial resolution than collicular neurons. (b) In a complex environment with many reflective objects, each echolocation call is followed by many object specific echoes. The IC can process each object specific echo which gives the bat a coarse overview on the scenario. The auditory cortex can process only one object specific echo with highest priority on the nearest object. However, the cortex gives the bat a detailed analysis of the object. (c) In a noisy environment, the echolocation system of bats can get masked. A call emission opens a time window of sensation which is closed by the subsequent acoustic signal (green bars indicate the extent of the time windows). During the time window, signals of conspecifics (violet bars) may be misinterpreted as echoes and close the window. Echo delay information has the lowest redundancy, when the bats equally distribute their call emission (no call grouping). However, low delay redundancy may not compensate information loss when call echo elements get jammed. This could result into a non-continuous delay representation and into a relatively large blind spot. In our example the longest jammed distance was 63 cm. This blind spot could be reduced by grouping the echolocation calls into units carrying redundant echo delays. However, no delay information is lost due to jamming because the bat could rely on one of the echoes deriving from one call group. The latter results into a more continuous delay representation where the blind spot was initially adjusted by the bat's intergroup distance of 51 cm. Collicular and cortical neurons can process delay information from the groups even right immediately after jamming. Thus, neuronal suppression can be overcome by the animals under these challenging echolocation situations.

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Selbstständigkeitserklärung

Ich erkläre hiermit, dass ich die Doktoarbeit selbstständig verfasst und keine anderen als die angegeben Quellen und Hilfsmittel benutzt habe.

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