A new animal model of paradoxical kinesia induced by 50-kHz ultrasonic vocalizations playback in rats: implications of the inferior colliculus

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SUMMARY

Motor impairments such as bradykinesia (slowness of movement) or akinesia (loss of movement) are among the most troubling symptoms seen in Parkinson's disease (PD) patients. PD patients exposed to visual or auditory stimuli might be able to exhibit normal motor responses, experiencing a phenomenon named paradoxical kinesia. Paradoxical kinesia is a sudden transient ability of akinetic patients to perform normal motor tasks. This phenomenon is known to depend on the patient's emotional state and external stimuli; however, the neural mechanisms underlying it are unknown. Here, a new animal model was developed (Study I) to investigate paradoxical kinesia by "awakening" cataleptic rats through presenting appetitive 50-kHz ultrasonic vocalizations (USV) which are typical for social situations with positive valence, like juvenile play or sexual encounters ("rat laughter"). Rats received systemic haloperidol to induce catalepsy which was assessed by means of the bar test. During that test, 50-kHz USV, 22-kHz USV or acoustic control stimuli were played back and compared to SILENCE. Only the 50-kHz USV was able to induce paradoxical kinesia in cataleptic rats. In addition, the role of the inferior colliculus (IC) was investigated in paradoxical kinesia induced by 50-kHz USV (Study II), since the IC not only serves as an acoustic relay station, but also modulates haloperidol-induced catalepsy. Glutamatergic and GABAergic neurotransmissions were selected, with rats receiving intracollicular NMDA, a glutamatergic agonist, or diazepam, a GABA/benzodiazepine agonist, 10 min before systemic haloperidol. During the catalepsy test rats were exposed to playback of 50-kHz USV and control stimuli. The results show that playback of 50-kHz USV induced paradoxical kinesia in rats which had systemically received haloperidol and vehicle into the IC. This paradoxical kinesia effect of 50-kHz USV playback on haloperidol-induced catalepsy was prevented by intracollicular NMDA administration. Although diazepam microinjected into the IC potentiated haloperidol-induced catalepsy, it did not affect the response to 50-kHz USV playback. Therefore, the NMDA receptor agonist suppressed the effectiveness of 50-kHz USV playback, whereas diazepam did not. These findings suggest that the IC is a key structure involved in paradoxical kinesia, with relevant processes being glutamatergic rather than GABAergic. This animal model thus appears useful for uncovering neural mechanisms of paradoxical kinesia and it might help to identify novel therapeutic targets for PD.

ZUSAMMENFASSUNG*

Motorische Beeinträchtigungen wie Bradykinesie (Langsamkeit der Bewegung) oder Akinesie (Bewegungsverlust) gehören zu den beunruhigendsten Symptomen bei Parkinson (PD) Patienten. PD-Patienten, die visuellen oder auditiven Reizen ausgesetzt sind, können möglicherweise normale motorische Reaktionen zeigen, wobei sie ein Phänomen erfahren, das paradoxe Kinesie genannt wird. Paradoxe Kinesie ist eine plötzliche, vorübergehende Fähigkeit von akinetischen Patienten, normale motorische Aufgaben auszuführen. Es ist bekannt, dass dieses Phänomen vom emotionalen Zustand des Patienten und von äußeren Reizen abhängt; die zugrundeliegenden neuralen Mechanismen sind jedoch unbekannt. In dieser Arbeit wurde ein neuartiges Tiermodell entwickelt (Studie I), um paradoxe Kinesien durch "Erwecken" kataleptischer Ratten durch die Präsentation appetitiver 50 kHz Ultraschallvokalisationen (USV) zu untersuchen, die typisch für soziale Situationen mit positiver Wertigkeit ("Lachen der Ratte") sind, wie Spielverhalten oder sexuelle Interaktionen. Die Ratten erhielten systemisches Haloperidol um Katalepsie zu induzieren, welche mittels des Bar-Tests beurteilt wurde. Während dieses Tests wurden 50-kHz-USV, 22-kHz-USV oder akustische Kontrollreize wiedergegeben und mit Stille verglichen. Nur die 50-kHz-USV konnte bei kataleptischen Ratten paradoxe Kinesien induzieren. Darüber hinaus wurde die Rolle des inferioren Colliculus (IC) in paradoxer Kinesie untersucht, die durch 50-kHz-USV induziert wurde (Studie II), da der IC nicht nur als akustische Relais-Station dient, sondern auch Haloperidol-induzierte Katalepsie moduliert. Glutamaterge und GABAerge Neurotransmission wurden ausgewählt, wobei die Ratten intracollikuläres NMDA, einen glutamatergen Agonisten oder Diazepam, einen GABA/Benzodiazepin-Agonisten 10 Minuten vor dem systemischem Haloperidol erhielten. Während des Katalepsietests wurden die Ratten einer Wiedergabe von 50 kHz-USV und Kontrollreizen ausgesetzt. Die Ergebnisse zeigen, dass die Wiedergabe von 50-kHz-USV paradoxe Kinesie bei Ratten induzierte, die systemisches Haloperidol und Vehikellösung in den IC erhalten hatten. Dieser paradoxe Kinesie-Effekt von 50-kHz-USV-Wiedergabe auf Haloperidol-induzierte Katalepsie wurde durch intracollikuläre NMDA-Verabreichung verhindert. Obwohl Diazepam, das in den IC injiziert wurde, die Haloperidol-induzierte Katalepsie potenzierte, beeinflusste es nicht die Reaktion auf 50 kHz-USV-Wiedergabe. Zusammengefasst unterdrückte der NMDA-Rezeptor-Agonist die Wirksamkeit der 50-kHz-USV-Wiedergabe, während Diazepam dies nicht tat. Diese Ergebnisse legen nahe, dass der IC eine Schlüsselstruktur ist, die in paradoxe Kinesien involviert ist, wobei die relevanten Prozesse eher glutamaterg als GABAerg sind. Dieses Tiermodell scheint daher nützlich zu sein, um neurale Mechanismen paradoxer Kinesie aufzudecken, und es könnte helfen, neue therapeutische Ziele für Parkinson Erkrankungen zu identifizieren.

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1 INTRODUCTION

1.1 Parkinson's disease

In 1817, the English surgeon James Parkinson was the first to describe "paralysis agitans" in his monograph entitled "*An Essay on the Shaking Palsy*", in which he detailed six patients with "involuntary tremulous motion with lessened muscular power, in parts not in action even when supported, with a propensity to bend the trunk forward and to pass from walking to a running pace" (Parkinson, 1817). Later on, studies between 1868 and 1881 made by the father of neurology, Jean Martin Charcot, were a landmark in the understanding of the disease, and he proposed, in honor of James Parkinson, that the syndrome should be named "*maladie de Parkinson*" (Parkinson's disease; Lees et al., 2009).

Parkinson's disease (PD) is the second most common neurodegenerative disorder (after Alzheimer's disease) and the most serious movement disorder (Hirtz et al., 2007). Considering the steady increase in the aging population, dysfunctional gait is observed in about one-third of the population above 70 years old (Verghese, 2006; Salzman, 2010). Among other symptoms such as cognitive impairments (Buracchio et al., 2010), idiopathic PD patients suffer mostly from movement impairments (Morris et al., 2001) such as bradykinesia (slowness of movement), tremor, limb rigidity and postural instability (Lees et al., 2009). Importantly, dysfunctional gait is commonly seen in PD patients, and is generally characterized by small steps (i.e., reduced stride length), lower cadence with festination and freezing of gait (FOG), in which the patient has difficulty in gait initiation or stopping when turning or approaching an obstacle (Giladi et al., 1992).

Most commonly, studies have reported FOG as a pure state of akinesia (Imai et al., 1986; Achiron et al., 1993; Giladi et al., 1997; Factor et al., 2002), characterized as an almost complete loss of movement (Schilder et al., 2017). In PD patients, akinesia is a symptom seen mostly at later stages of the illness (Morris et al., 2001; Jankovic, 2008). Furthermore, akinesia can also be seen in response to drug toxicity, high-dosed neuroleptics, such as haloperidol, or in other neurological diseases, in particular multiple system atrophy (Wenning et al., 2004), progressive lacunar cerebro-sclerosis, or post-encephalitis (Satterthwaite et al., 2008; Schilder et al., 2017).

The cause of PD remains as elusive as when it was described in 1817, although neuropathological clues are now better understood (Lees et al., 2009). The symptoms seen in PD are caused by the region-specific selective loss of dopaminergic neurons from the pars compacta of the substantia nigra (SNpr; Lees et al., 2009). Cardinal motor symptoms in PD can be alleviated by pharmacological treatment such as L-dopa, dopamine (DA) agonists, monoamine oxidase type B (MAO-B) inhibitors (Pahwa and Lyons, 2014) and deep-brain stimulation (Sharma et al., 2012). However, the beneficial effects of these treatments on motor dysfunctions are typically limited and decrease over time (Blin et al., 1990; Grabli et al., 2012).

Interestingly, clinical evidence has shown that auditory stimulation via rhythmic cues can be used successfully in the rehabilitation of motor function in patients with motor disorders, such as PD (Thaut et al., 2010; Lim et al., 2005; Spaulding et al., 2013). This approach is noninvasive, cost-efficient and easily applicable. Patients with PD that are exposed to auditory stimulation, such as repeated isochronous sound (i.e., metronome) or music with a salient beat structure, generally walk faster, increase step length and tend to walk without showing akinesia episodes (De Bruin et al., 2010; Arias and Cudeiro, 2010). Remarkably, this akinesia depends on the emotional state of the subject and certain external stimuli (Jankovic, 2008). Namely, patients suffering from akinesia might overcome such a state when exposed to auditory or visual stimuli, therefore experiencing a phenomenon called "Paradoxical Kinesia" (Ballanger et al., 2006; Jankovic, 2008).

1.2 Paradoxical Kinesia

A phenomenon termed by the French neurologist Achille Alexandre Souques in 1921, paradoxical kinesia was first described as "a sudden and brief period of mobility typically seen in response to emotional or physical stress". In definition, paradoxical kinesia is a sudden transient ability of akinetic patients to perform motor tasks they are otherwise unable to perform. This enigmatic phenomenon is mostly observed in advanced stages of PD (Glickstein and Stein, 1991). For instance, akinetic parkinsonian patients, when properly stimulated by visual or auditory stimuli, can be able to perform tasks, such as kicking a tennis

ball (Asmus et al., 2008), riding a bicycle or running, which they were otherwise unable to perform (Glickstein and Stein, 1991).

There are several well-documented examples of paradoxical kinesia. Martin (1967) encountered that a visual stimulus such as transversely oriented stripes along the path elicited an improvement in walking. Importantly, this improvement was seen independently of medication. Furthermore, Anzak et al. (2011) have shown that a loud (96 dB) auditory stimulus in addition to a visual cue given at the same time to patients with PD improved peak rate of force development and the magnitude of force developed when patients were asked to grip a dynamometer as quickly and strongly as possible. Interestingly, the patients included in this experiment were tested whilst "off" and "on" dopaminergic medication, the results associated with improved motor performance in PD were independent of dopaminergic state. Nevertheless, some researchers claim that paradoxical kinesia only occurs under influence of medication (Hardie, 1990).

Conversely, life-threatening events such as the sound of a car accident (Daroff, 2008), the sensation of an earthquake (Bonanni et al., 2010) or the sight of a fire (Glickstein and Stein, 1991) may also trigger paradoxical kinesia in PD patients. However, paradoxical kinesia is not restricted to stressful or even life-threatening events, since familiar music can also induce paradoxical kinesia in patients (Sacks, 1973; Rubinsten et al., 2002). In fact, Oliver Sacks was probably the first to note that familiar music can induce paradoxical kinesia, i.e. "temporary awakenings", in patients with severe akinesia. Since then, many clinical studies have shown that music or cueing sounds can be therapeutically valuable for PD patients to treat akinesia (Glickstein and Stein, 1991; Clark et al., 2009; Anzak et al., 2011).

In general, patients with PD might have intact motor programs but have difficulty accessing them without external sensory stimulation (Jankovic, 2008; Clark et al., 2009). It is possible that this intriguing phenomenon may activate neural systems that can override parkinsonian impairment (Anzak et al., 2011). Uncovering the neural mechanisms of paradoxical kinesia might yield more effective therapies for motor impairment in PD. However, since researching the neural mechanisms in humans is somehow limited, the usage of animal models is necessary. In this manner, animal models of paradoxical kinesia might facilitate the understanding of the neural mechanisms underlying this phenomenon which are largely unknown.

1.3 Animal Models of Paradoxical Kinesia

In order to investigate paradoxical kinesia in rats, firstly motor impairment has to be induced. Systemic or intrastriatal administration of haloperidol acts mainly by blocking striatal post-synaptic dopaminergic D2 receptors therefore inducing akinesia (Hornykiewicz, 1973; Wadenberg et al., 2001) which is commonly studied as catalepsy in rodents. Catalepsy is a state of immobility in which the animals are unable to correct externally imposed postures; this state mimics the lack of spontaneous motor activity that is commonly seen in some PD patients (Sanberg, 1980).

So far, there are only a few studies showing that cataleptic rats can yield motor activity when exposed to an external stressor and consequently experience paradoxical kinesia. For instance, a study performed by Yntema and Korf (1987) showed that environmental stress or emotional stress can decrease the catalepsy caused by haloperidol. Similarly, Clark et al. (2009) reported that rats which had received haloperidol-induced catalepsy exhibited motor improvement when exposed to external auditory stimulation (key jingled or chip bag crumpled). Moreover, Brown et al. (2010) exposed cataleptic rats to familiarized acoustic stimulation and, as a result of that, rats had reduced catalepsy time. These results suggest that paradoxical kinesia might be reproduced in rats through environmental stress or via acoustic stimulation.

Along that line, some evidence indicates that the auditory system might be involved in the paradoxical kinesia induced by acoustic stimulation. Some studies have shown that the inferior colliculus (IC), a structure responsible for auditory processing, participates in the regulation of motor activity. In turn, the microinjection of the glutamate receptor antagonist MK-801 into the IC significantly reduced the catalepsy induced by systemic or intrastriatal haloperidol injection (Melo et al., 2010; Medeiros et al., 2014). Likewise, the catalepsy induced by the N(G)-nitro-L-arginine (L-NOARG), an inhibitor of enzyme nitric oxide synthase (NOS), was reduced by an intracollicular administration of another glutamate receptor antagonist AP7 into the IC (Jacopucci et al., 2012). In addition, Melo-Thomas and Thomas (2015) have shown that high frequency electrical deep brain stimulation can improve motor impairment induced by haloperidol in rats and proposed that deep brain stimulation at

the IC level can be an animal model of paradoxical kinesia. Therefore, these data substantially support the role of the IC in paradoxical kinesia.

1.4 The Inferior Colliculus

1.4.1 Projections of the IC

The IC is a midbrain structure implicated in auditory processing; in a rat's brain, the IC is anatomically divided into a central nucleus, external and dorsal cortex. The IC is positioned in the central auditory system, integrating input from a broad range of auditory brainstem nuclei and relaying information to the auditory cortex through the auditory thalamus pathway (Marsh et al., 2002). The latter is known as the main auditory thalamic relay, projecting from the IC to the medial geniculate nucleus and thus, to the auditory and premotor cortex (Cappe et al., 2009).

The major output of the IC is to the auditory thalamocortical system, however, via separate pathways, the IC receives crossed input from the opposite IC, ascending input from a number of auditory nuclei in the lower brainstem and also receives descending input from the auditory cortex. These connections suggest that the IC integrates information from various auditory sources. In addition, these connections raise the possibility that sensory processing in the IC is modulated by motor action and also that the midbrain integrates somatosensory information (Casseday et al., 2002).

Furthermore, the IC is distinguished from other auditory centers in the brainstem by its connections with motor systems (Casseday and Covey, 1996). Particularly, there are non-auditory inputs to the IC, for instance, projections from the amygdala (Marsh et al., 2002) and also projections which are considered to be part of the motor systems, such as from the SNpr (Olazábal and Moore, 1989) and the globus pallidus (Moriizumi and Hattori, 1991) to the IC. The IC also transmits information to motor systems such as the deep superior colliculus, and the cerebellum, via the pontine gray (Casseday et al., 2005). Moreover, these connections propose that the IC is not only responsible for processing auditory information and sending it to higher auditory centers but also modulates motor action in a direct fashion. In short, the

IC is ideally suited to process auditory information based on behavioral context and to direct information for guiding action in response to this information (Aitkin 1986; Casseday and Covey 1996).

1.4.2 The functionality of the inferior colliculus

In order to understand the function of the IC, it is particularly important to view its physiology within the behavior context. In all mammals, the auditory system plays a basic role in identifying sounds, selectively activating neural systems that focus attention on sounds and generating suitable motor responses (Malmierca, 2006). The IC contains a high density of GABA receptors (Oliver et al., 1994) as well as other receptors such as NMDA, AMPA, glycine, serotonin (Huerly and Sullivan, 2018) and somatostatin (Wynne and Robertson, 1997). Apart from the fact that the IC is responsible for processing auditory information, it also plays an important role in modulating motor behavior such as haloperidol-induced catalepsy. Interestingly, in the IC glutamatergic and GABAergic mechanisms are the two types of neurotransmitters involved in the regulation of haloperidol-induced catalepsy in rats (Melo et al., 2010; Tostes et al., 2013; Medeiros et al., 2014).

Previously, the IC has been mostly investigated for its role in activating behavioral defense reactions. Researchers have shown that defensive behavioral responses, such as freezing and escape, are mediated by NMDA mechanisms (Cardoso et al., 1994; Brandão et al., 1999; Nobre et al., 2004) or by GABAergic mechanisms in the IC (Brandão et al., 1988, 1993; Melo et al., 1995). Moreover, electrical stimulation of the IC also causes behavioral activation together with autonomic reactions usually observed as part of the defense responses (Brandão et al., 1988; Melo et al., 1995; Maisonnette et al., 1996; Troncoso et al., 2003). Overall, for decades many studies have been investigating the functionality of the IC on aversive responses, however, the stimulation or inhibition of the IC via glutamatergic and GABAergic mechanisms has demonstrated that the IC plays an imporant role in paradoxical kinesia in rats (Melo et al., 2010; Tostes et al., 2013; Medeiros et al., 2014; Melo-Thomas and Thomas, 2015).

Accordingly, Melo et al. (2010) carried out a further study to better understand how the IC modulates catalepsy induced by haloperidol in rats. Interestingly, they have shown that

intracollicular microinjections of glutamatergic drugs can modulate haloperidol-induced catalepsy. Specifically, administration of the NMDA glutamate receptor antagonist MK-801 into the IC significantly reduced catalepsy time, whereas the agonist NMDA potentiated it. In addition, another study showed that intracollicular microinjection of the GABAergic agonist midazolam potentiated haloperidol-induced catalepsy whereas the GABAergic antagonist bicuculline produced a biphasic effect (Tostes et al., 2013). Most recently, evidence indicates that catalepsy induced by haloperidol can be reduced by high frequency electrical deep brain stimulation in the IC, representing an animal model of paradoxical kinesia induced by aversive stimulation, since this stimulation led to flight responses in rats (Melo-Thomas and Thomas, 2015).

Taking into consideration that an auditory structure, such as the IC, can modulate paradoxical kinesia in rats and that in humans this phenomenon can be induced not only by aversive auditory stimuli (Daroff, 2008) but also by appetitive ones (Rubinsten et al., 2002), the usage of rat's ultrasonic vocalizations (USV) might provide an interesting approach to study paradoxical kinesia in rats induced by auditory stimuli.

1.4.3 Rat's ultrasonic vocalizations

The typical hearing range of a human is between 20-Hz and 20-kHz, however, rats generally vocalize above the human hearing threshold, at 20 kHz to 100 kHz (Portfors, 2007). Depending on environmental factors, age and subject's current state, rats emit distinct types of USV (Knutson et al., 2002; Portfors, 2007). USV are a prominent component of the behavioral repertoire displayed by rats and serve important communicative functions as situation-dependent socio-affective signals (Brudzynski, 2013; Wöhr and Schwarting, 2013). For instance, juvenile and adult rats produce a complex repertoire of high-frequency vocalizations named 50-kHz USV, a form of "rat laughter" (Panksepp, 2005), which are mostly observed in anticipation of or during naturalistic rewarding situations such as rough-and-tumble play (Knutson et al., 1988; Brunelli et al., 2006; Burgdorf et al. 2008), tickling (Burgdorf and Panksepp, 2001; Schwarting et al. 2007; Burgdorf et al. 2007; Ishiyama and Brecht, 2016), mating (Burgdorf et al. 2008; White and Barfield 1990), social contact (Burgdorf et al., 2008; White and Barfield, 1987), food consumption (Burgdorf et al., 2000), electrical self-stimulation of the brain (Ishiyama and Brecht, 2016; Burgdorf et al., 2000; Burgdorf et al., 2007) and addictive drugs (Knutson et al., 1999; Burgdorf et al., 2001).

On the other hand, juvenile and adult rats emit low frequency vocalizations, termed 22-kHz USV, which are considered to be part of the animal's defensive repertoire and are mostly seen in aversive situations (Brudzynski and Holland, 2005). Rats emit 22-kHz USV in various aversive contexts such as during confrontation with predators (Blanchard et al., 1991), inter-male aggression (Sales, 1972), the refractory period that follows ejaculation (Barfield and Geyer, 1972), social isolation (Brunelli et al., 2006), drug withdrawal (Covington and Miczek, 2003), foot-shocks (Wöhr et al., 2005; Parsana et al., 2012) and single touch of a human hand on rats placed in an unfamiliar environment (Brudzynski and Ociepa, 1992). Apparently, 22-kHz USV reflect a negative affective state and they are known as "alarm cries" (Blanchard et al., 1991) and have been thought to signal the intention of withdrawal from ongoing social activities (Brudzynski, 2013).

Overall, playback studies have shown that rats respond differently when exposed to either 50-kHz USV or 22-kHz USV. In other words, playback of 50-kHz USV leads to social approach behavior in the recipient and behavioral activation towards the sound source (Wöhr and Schwarting, 2007; Engelhardt et al., 2017), whereas playback of 22-kHz USV leads to behavioral inhibition and activation of the fight/flight/freezing system (Wöhr and Schwarting, 2007; Parsana et al., 2012). Moreover, the usage of playback USV seems to be a powerful tool to investigate natural reward circuits in the brain (Burgdorf and Panksepp, 2006), emotion and motivation aspects in rodents (Wöhr and Schwarting, 2007; Wöhr and Schwarting, 2013).

Hence, assuming that acoustic stimulation can produce paradoxical kinesia in humans and that the IC has a regulatory role of haloperidol-induced catalepsy in rats and also relays auditory information, it seems relevant to study the IC functionality within the behavior context using the USV playback as an external auditory stimulation in rats.

2 GENERAL OVERVIEW

OBJECTIVES, HYPOTHESES AND METHODS

2.1 Study I - Awakenings in rats by ultrasounds: a new animal model for paradoxical kinesia

Although bradykinesia (slowness of movement) and akinesia (loss of movement) are typical features of patients with Parkinson's diseases (Ballanger et al., 2006), it has been suggested that these patients are able to produce normal motor responses in the context of urgent or externally driven situations, experiencing a phenomenon called paradoxical kinesia (Souques, 1921). Paradoxical kinesia is a sudden and brief period of mobility which can be seen, for instance, in response to an emotional auditory stimulus, such as music (Sacks, 1973) or in response to an aversive context, such as circumstances of war (Schlesinger et al., 2007) or the sound of a car accident (Daroff, 2008). The mechanisms underlying paradoxical kinesia are unknown due to a paucity of valid animal models that faithfully reproduce this phenomenon. The USV playback technique is an interesting approach to investigate socio and communicative functions in rats and can also be used as an appetitive or aversive auditory stimulus (Wöhr and Schwarting, 2007).

In this manner, the fundamental purpose of the first part of Study I is to induce paradoxical kinesia in rats using an emotionally and motivationally relevant appetitive acoustic stimulus: the playback of 50-kHz USV; in the second part, the aim is to induce paradoxical kinesia in rats using an aversive acoustic stimulus: the playback of 22-kHz USV. It is expected that both stimuli (50-kHz USV or 22-kHz USV) induce paradoxical kinesia in cataleptic rats.

2.1.1 Experiment I - Method

Rats received haloperidol intraperitoneally (0.5 mg/kg) and after 60 min they were brought to the catalepsy test. The test consisted of gently placing the rat with its forepaws on a horizontal bar and measuring the time until it stepped down from the bar with both forepaws (maximum 300s). Rats were tested in four different periods of time and in each one of them the acoustic stimuli started 30s after the animal was placed on the bar and were presented for

270s (total test duration: 30s + 270s = 300s), followed by an inter-stimulus interval of 300s. The 50-kHz USV, white-noise, background noise and silence were presented in a random order (for details see Tonelli et al., 2018a).

2.1.2 Experiment II - Method

To test the effects of 22-kHz USV in cataleptic animals, the bar test was performed during which a given rat was exposed to different playback presentations of (i) 22-kHz USV, (ii) phase-scrambled and frequency-shifted 22-kHz USV (22-kHz USV CONTROL) and (iii) SILENCE. Since it is assumed that the motivational impact of 22-kHz USV may depend on experience, namely to perceive them as aversive, half of the rats underwent an auto-conditioning procedure before the playback test. Therefore, we had to establish a new paradigm in which rats would vocalize 22-kHz USV and learn by themselves its meaningfulness. In order to auto-condition rats in vocalizing 22-kHz USV, they were individually placed in a shock chamber where they received from 3 to 5 unsignaled foot shocks (0.8 mA, 0.5s) with an inter-trial interval of 180s (adapted from Parsana et al., 2012). Hence, the 22-kHz USV was recorded and played back to the same group of animals in another context (for details see Tonelli et al., 2018a).

2.2 Study II - Paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats depends on glutamatergic mechanisms in the inferior colliculus.

The IC represents a prime candidate target to investigate paradoxical kinesia mechanisms induced by the appetitive 50-kHz USV playback, since it not only relays auditory information but also modulates via glutamatergic and GABAergic mechanisms the catalepsy induced by haloperidol in rats (Melo et al., 2010; Tostes et al., 2013; Medeiros et al., 2014). To this aim, in Study II, the main objective is to uncover possible mechanisms underlying paradoxical kinesia by investigating the role of glutamatergic and GABAergic substrates in the IC during the presentation of appetitive 50-kHz USV playback. Overall, the hypotheses are that:

a) The microinjection of the glutamatergic receptor agonist NMDA into the IC potentiates the catalepsy induced by haloperidol in rats;

b) Considering the potentiation effect on catalepsy time of the microinjection of NMDA into the IC, it is expected that the intracollicular microinjection of NMDA in rats which had received haloperidol suppresses the paradoxical kinesia induced by the appetitive 50-kHz USV playback and does not affect the integrity of the lower auditory system;

c) The microinjection of the diazepam, a GABA/benzodiazepine (BZD) receptor agonist, into the IC potentiates the catalepsy induced by haloperidol;

d) Taking into consideration the potentiation effect on catalepsy time of the microinjection of diazepam into the IC, it is expected that the intracollicular microinjection of diazepam in rats which had received haloperidol suppresses the paradoxical kinesia induced by the appetitive 50-kHz USV playback and does not affect the integrity of the lower auditory system;

2.2.1 Experiment I A – Method

Rats received a microinjection of the agonist NMDA (30nmol/0.5µl) or saline into the IC and 10 min later haloperidol (0.5 mg/kg) intraperitoneally. The catalepsy test was performed at 20, 40, 60, 80 and 100 min after intracollicular administration (for details see Tonelli el al., 2018b).

2.2.2 Experiment I B - Method

In order to investigate the role of the IC in paradoxical kinesia induced by appetitive 50-kHz USV, rats received a microinjection of the glutamatergic receptor agonist NMDA (30nmol/0.5µl) or saline into the IC and 10 min later haloperidol (0.5 mg/kg) intraperitoneally. Approximately 50 min later the catalepsy test was performed and rats were exposed to the acoustic stimuli in the following order: Silence, 50-kHz USV, white-noise and background noise with an inter-stimulus interval of 300s. The experimental design was the same as described in Study I (Experiment – I) with an exception that rats were tested during catalepsy for 600 seconds (for details see Tonelli el al., 2018b).

2.2.3 Experiment II A - Method

We further explored the role of the IC in the paradoxical kinesia induced by appetitive 50-kHz USV playback in rats by microinjecting diazepam, a GABA/BZD agonist, into this structure. Here, two different doses of diazepam (10µg/0.5µl or 20µg/0.5µl) or vehicle were tested in combination with haloperidol injected intraperitoneally. The catalepsy test was performed at 20, 40, 60, 80 and 100 min after intracollicular administration (for details see Tonelli el al., 2018b).

2.2.4 Experiment II B - Method

In addition, the question addressed was whether the appetitive 50-kHz USV would induce paradoxical kinesia in rats receiving diazepam in the IC. Rats received a microinjection of diazepam (10µg/0.5µl or 20µg/0.5µl) or vehicle into the IC and 10 min later a systemic injection of haloperidol (0.5 mg/kg). Approximately 50 min after haloperidol injection the catalepsy test was performed and rats were exposed to the acoustic stimuli in the following order: Silence, 50-kHz USV, white-noise and background noise with an inter-stimulus interval of 300s (for details see Tonelli el al., 2018b).

3 SUMMARY OF PUBLICATIONS

3.1 Study I: Awakenings in rats by ultrasounds: a new animal model for paradoxical kinesia

Tonelli, L.C., Wöhr, M., Schwarting, R.K., Melo-Thomas, L, Awakenings in rats by ultrasounds: A new animal model for paradoxical kinesia. **Behav Brain Res**. 337 (2018) 204–209.

Summary

Paradoxical kinesia refers to a sudden transient ability of akinetic patients to perform motor tasks they are otherwise unable to perform. The mechanisms underlying this phenomenon are unknown due to a paucity of valid animal models that faithfully reproduce paradoxical kinesia. Here, in a first experiment, we present a new method to study paradoxical kinesia by "awakening" cataleptic rats through presenting appetitive 50-kHz ultrasonic vocalizations (USV), which are typical for social situations with positive valence, like juvenile play or sexual encounters ("rat laughter"). Rats received systemic haloperidol to induce catalepsy, which was assessed by means of the bar test. During that test, 50-kHz USV, time- and amplitude-matched white noise (NOISE), or background noise (BACKGROUND) were played back and compared to SILENCE. Every animal was exposed to all four acoustic stimuli in random order, with four independent groups of rats being tested. Only when exposed to playback of appetitive 50-kHz USV, the otherwise akinetic rats rapidly started to move efficiently. The acoustic control stimuli, in contrast, did not release rats from catalepsy, despite eliciting the auditory pinna reflex and head movements towards the sound source. Moreover, in a second experiment, playback of aversive 22-kHz USV and relevant acoustic control stimuli did also not significantly affect catalepsy time. Together, our animal model provides a completely new approach to study mechanisms of paradoxical kinesia, which might help to improve behavioral therapies for Parkinson's disease and other disorders, where akinetic or cataleptic states occur.

3.2 Study II: Paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats depends on glutamatergic mechanisms in the inferior colliculus.

Tonelli, L.C., Wöhr, M., Schwarting, R.K., Melo-Thomas, L, Paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats depends on glutamatergic mechanisms in the inferior colliculus. **Neuropharmacology.** 135 (2018) 172 – 179.

Summary

Paradoxical kinesia is a sudden transient ability of akinetic patients to perform motor tasks they are otherwise unable to perform. This phenomenon is known to depend on the patient's emotional state and external stimuli. Paradoxical kinesia can be induced by appetitive 50-kHz ultrasonic vocalizations (USV) in rats displaying catalepsy following systemic haloperidol. We investigated the role of the inferior colliculus (IC) in paradoxical kinesia induced by 50-kHz USV, since the IC modulates haloperidol-induced catalepsy. We focused on glutamatergic and GABAergic neurotransmission, with male rats receiving intracollicular NMDA or the GABA receptor agonist diazepam 10 min before systemic haloperidol. Catalepsy time was assessed by means of the bar test, during which rats were exposed to playback of 50-kHz USV, white noise, and background noise. Our results show that playback of 50-kHz USV induced paradoxical kinesia by reducing haloperidol-induced catalepsy in rats which had received saline intracollicular microinjection. This paradoxical kinesia effect of 50-kHz USV playback on haloperidol-induced catalepsy was prevented by intracollicular NMDA administration. Although intracollicular diazepam microinjection potentiated haloperidol-induced catalepsy, it did not affect the response to 50-kHz USV playback. Together, NMDA receptor agonist suppressed the effectiveness of 50-kHz USV playback, whereas diazepam did not. These findings suggest that the IC is a key structure involved in paradoxical kinesia, with relevant processes being glutamatergic rather than GABAergic. Our approach thus appears useful for uncovering neural mechanisms of paradoxical kinesia and it might help identifying novel therapeutic targets for Parkinson's disease.

4 GENERAL DISCUSSION

4.1 Appetitive 50-kHz USV induce paradoxical kinesia in cataleptic rats

Primordially, it is important to highlight that there is a paucity of animal studies using familiar or appetitive auditory stimuli to improve motor impairment in rats. Previously, Clark and colleagues (2009) used spectrographically undefined sounds (like key jingles) and simple righting responses to restore balance in haloperidol-treated rats. In another study, Brown et al. (2010) have shown that acoustic tones reduced akinesia under haloperidol conditions, but first rats underwent substantial prior training to respond to the tones accordingly. Overall, in both studies, rats had to be previously trained and unfamiliar and unnatural auditory stimuli were used.

Conversely, in Study I, a natural, familiar acoustic stimulus was used which is spectrographically well-characterized and contains ethologically valid signals (50-kHz USV playback). When exposed to the 50-kHz USV playback, rats showed rather complex approach responses. Namely, even though the rats were under influence of haloperidol, they were able to localize the sound source, orienting themselves, stepping down from the bar, and thus coordinate locomotion towards the active ultrasonic speaker. Furthermore, we have shown that these effects require specific acoustic features, since 50-kHz call sequences but not time- and amplitude-matched white noise were effective in reversing catalepsy, indicating that mere arousal is not sufficient for this outcome. The results seen in response to playback of 50-kHz USV are consistent with previous findings in un-drugged rats, where we showed that playback of such 50-kHz USV, but not various control stimuli, induces locomotion and approach behavior (Wöhr and Schwarting, 2007), which highlights their motivational relevance as social signals in rats. To the best of my knowledge, this is the first time that appetitive 50-kHz USV has been used as an external trigger to induce paradoxical kinesia in rats.

Interestingly, humans experience the phenomenon paradoxical kinesia when exposed to a familiar sound (Rubinstein et al., 2002; Jankovic, 2008; Arias and Cudeiro, 2008). This response, amelioration of motor impairments, might be linked to the meaningfulness of the

sound (Distler et al., 2016) which may be relevant here since the ultrasonic signals are part of the rats' communicative repertoire (Wöhr and Schwarting, 2007; Brudzynski, 2013), i.e. they fulfill the requirements of familiarity and meaningfulness. Besides, paradoxical kinesia can be induced by external stimuli through "energizing" relevant action systems in the brain (Ballanger et al., 2006) which are otherwise insufficiently activated. Thus, in Study I (Tonelli et al., 2018a), the motivational properties of 50-kHz USV playback may activate such relevant systems in the rats' brain.

4.2 Acoustic control stimuli have no effect on catalepsy time in rats

In order to rule out the hypothesis that any other playback acoustic stimuli might induce paradoxical kinesia, rats were exposed to the presentation of other acoustic control stimuli, but only playback of 50-kHz USV was efficient in releasing rats from haloperidol-induced catalepsy. This evidence shows that regardless which control stimuli (NOISE or BACKGROUND) was presented, catalepsy time was not affected when compared with SILENCE, suggesting that the response to 50-kHz USV is not merely because it is a sound, but due to its meaningfulness.

Moreover, cataleptic rats stepping down from the bar was not the only striking factor; rats, when exposed to 50-kHz USV playback, were driven by the sound source, i.e. only rats exposed to 50-kHz USV explored more the proximal zone of the arena, in which the active ultrasonic speaker was placed, than the distal part containing the inactive ultrasonic speaker. Rats rarely stepped down during the control stimuli, and when they did they had no preference in exploring the arena. Thus, these facts support the notion that it is only when exposed to the 50-kHz USV playback that rats are released from catalepsy and able to display approach behavior. Interestingly, immediately after the 50-kHz USV playback ended, rats displayed catalepsy again. This event resembles what it is known about PD patients (Rubinstein et al., 2002; Jankovic, 2008; Arias and Cudeiro, 2008) when they return to an akinesia state after an auditory or visual stimulus has induced paradoxical kinesia, strongly supporting the face validity of the animal model presented in this dissertation.

4.3 Rats' ability to hear is not affected by haloperidol

It is important to mention the fact that rats were released from catalepsy specifically in response to 50-kHz USV, however, their response (not stepping down from the bar) to the control stimuli was not due to an inability to hear sounds. Rats have an acoustic startle reflex, which is required in response to auditory stimuli (Sinex et al., 2001). One of the components of this reflex, the auricular reflex, promotes pinna movements, which is caused by acoustic stimulation. This pinna reflex triggers a number of motor responses throughout the whole body (Li and Frost, 2000). Altogether, these motor responses to acoustic stimuli play a crucial role in survival by increasing selective attention (Landis and Hunt, 1939). In this particular case, rats when exposed to either playback of 50-kHz, NOISE or BACKGROUND, displayed pinna reflex, followed immediately by head movements towards the source of the sound. Expectedly, rats showed neither pinna reflex nor head movements during the SILENCE test. According to Horta-Júnior et al. (2008), these results confirm the integrity of the lower auditory systems to process all the acoustic stimuli.

4.4 Aversive 22-kHz does not elicit paradoxical kinesia in cataleptic rats

In humans, paradoxical kinesia is also observed when aversive situations occur. Particularly, life-threatening events such as war circumstances (Schlesinger et al., 2007), the sound of a car accident (Daroff, 2008), the sensation of an earthquake (Bonanni et al., 2010a) and the sight of a fire (Glickstein and Stein, 1991) can induce paradoxical kinesia in PD. Here, the aversive 22-kHz USV is thought to be part of the rats' communicative repertoire and might be perceived as life-threatening, i.e. playback of 22-kHz USV induce freezing behavior in rats. However, in contrast to 50-kHz USV, playback of 22-kHz USV was not effective in reducing haloperidol-induced catalepsy in rats, even though in half of the subjects, their motivational properties to perceive 22-kHz USV playback were enhanced by prior auto-conditioning (Parsana et al., 2012).

This finding was contrary to the hypothesis presented in Study I. At first sight, this result might indicate that these aversive acoustic stimuli are not effective in the cataleptic state, which contrasts with the findings observed in PD patients (for review see Banou, 2015)

and to a previous study in rats, showing that a high frequency electrical deep brain stimulation applied at the IC level, which is aversive, induced paradoxical kinesia in rats (Melo-Thomas and Thomas, 2015).

Regarding findings from Study I, it seems relevant to point out the fact that a typical response to playback of 22-kHz USV is reduced activity or transient immobility (Wöhr and Schwarting, 2010), which might not be compatible with the current model of testing haloperidol-induced catalepsy. Therefore, the hypothesis that 22-kHz USV may possibly reduce haloperidol-induced catalepsy should not be completely excluded. Alternatively, one possible way to better investigate this hypothesis would be to establish an active flight response to 22-kHz USV before testing their effects on the cataleptic state.

4.5 Animal model of Paradoxical Kinesia using the appetitive 50-kHz USV

Since 2007 our research group has taken a step forward to better understand how rats perceive ultrasonic communication. Playback studies have become essential to investigate motivational behavior aspects and emotion components in rodents (Wöhr and Schwarting, 2007; Wöhr and Schwarting, 2013). Uniquely, the playback of appetitive 50-kHz USV evokes motor responses in rats, such as social approach behavior in the recipient and behavioral activation towards the ultrasonic sounds (Wöhr and Schwarting, 2007; Engelhardt et al., 2017; Tonelli et al., 2018a). In this dissertation, despite possible limitations regarding the aversive playback of 22-kHz USV, the phenomenon of paradoxical kinesia seen in akinetic rats in response to playback of 50-kHz USV resembles that observed in PD patients in clinics (Sacks, 1973, Thaut et al., 1996; Rubinstein et al., 2002; Arias and Cudeiro, 2008; Sihvonen et al., 2017).

Furthermore, it is valid to emphasize some advantages of this new animal model of paradoxical kinesia over existing ones. Principally, familiar and meaningful ethologically valid signals are applied, which are precisely defined, and between-subject variance can easily be minimized due to the present playback approach, with all subjects being exposed to the exact same stimulus. To the best of my knowledge, this paradigm is the first to use a natural appetitive acoustic stimulus to induce paradoxical kinesia in cataleptic rats. Although other researchers have succeeded in investigating paradoxical kinesia in rats (Clark., et al., 2009;

Brown et al., 2010), they did not use rats' natural familiar auditory stimulation. Clark and coworkers (2009), jingled a set of keys or crumpled a chip bag to produce auditory stimulation for rats. Moreover, Brown et al. (2009), rewarded rats with sucrose pellets upon acoustic cue presentations, thus the catalepsy induced by haloperidol was reduced in rats exposed to this acoustic cueing. Another study, conducted by Yntema and Korf (1987), investigated whether environmental stress or emotional stress would affect the expression of catalepsy caused by haloperidol. They showed that prior environmental stress such as forced immobilization (gauze bandage wrapping), exposure to cold and handling (continuously moved hand to hand for 20 min) reduced the catalepsy induced by haloperidol.

Importantly, the examples cited above required prior training, however, the animal model presented in this dissertation required no training since the response elicited by 50-kHz USV is an unconditioned one. Last but not least, this new paradigm allows the study of paradoxical kinesia without exposure to aversive stimuli, which might be most appropriate to study how pleasant and appetitive stimuli exert their promotive effects in akinetic or cataleptic human subjects. Furthermore, the fact that rats are released from catalepsy in response to an emotionally and motivationally relevant appetitive auditory stimulus, but become cataleptic again immediately after playback is turned off, mimics findings on paradoxical kinesia in humans, and hence supports the model's face validity.

4.6 How might the inferior colliculus be involved in paradoxical kinesia?

The mechanism underlying the phenomenon of paradoxical kinesia in humans is yet to be fully understood. Certainly, the usage of animal models to study this phenomenon has become relevant in order to discover brain mechanisms linked to it. In animals, with regard to the addressed question, firstly one has to consider the fact that the IC is the main site of auditory integration at the midbrain level and also represents a major output to premotor pathways that regulate evoked motor behavior (Casseday et al., 2002). For example, the IC has connections with the motor systems such as inputs from the SNpr (Olazábal and Moore, 1989) and from the globus pallidus (Moriizumi and Hattori, 1991). Secondly, besides the fact that the IC processes auditory information, it can also modulate (via glutamatergic and GABAergic receptors) the catalepsy induced by haloperidol (Melo et al., 2010; Tostes et al., 2013; Medeiros et al., 2014), which mainly acts by blocking dopaminergic D2 receptors in the

striatum where they can generally be found on GABAergic projection neurons and cholinergic interneurons (Johnson et al., 2014; Kharkwal et al., 2016).

Along that line, the results of study II (Tonelli et al., 2018b) show that intracollicular administration of the glutamate receptor agonist NMDA potentiated haloperidol-induced catalepsy in rats, corroborating with previous results (Melo et al., 2010; Medeiros et al., 2014). They strengthen the assumption that glutamate-mediated mechanisms in the neural circuits at the IC level can influence a motor impairment induced by impaired nigrostriatal DAergic neurotransmission. More importantly, Study II (Tonelli et al., 2018b) shows for the first time that GABA/BZD receptor agonist diazepam microinjected into the IC potentiated the catalepsy induced by systemic haloperidol in rats. This result sustains previous experiments demonstrating that haloperidol-induced catalepsy can be potentiated by intracollicular administration of midazolam, another type of GABA/BZD receptor agonist (Tostes et al., 2013). Although the opposite effect of GABAergic and glutamatergic agonists would be expected, these apparently contradictory results may be explained by activation of different projections. For instance, the IC sends direct glutamatergic projections to pontine nuclei (ponto cerebellar auditory pathway; Saint Marie, 1996) and GABAergic and glutamatergic projections to the medial geniculate body (MGB; Winer et al., 1996; Peruzzi., 1997). Therefore, stimulation of these projections may underlie temporal patterns of inhibition and excitation.

Conversely, antagonizing NMDA or GABA receptors in the IC generates different responses in cataleptic rats. In previous studies, it has shown that systemic or intrastriatal haloperidol-induced catalepsy can be significantly reduced by prior microinjection of the NMDA glutamate receptor antagonist MK-801 into the IC (Melo et al., 2010; Medeiros et al., 2014). Moreover, intracollicular microinjection of the GABAergic antagonist bicuculline can produce a biphasic effect, from attenuation to potentiation of catalepsy induced by systemic haloperidol (Tostes et al., 2013). Together, these results point to the IC as an important sensorimotor interface influencing haloperidol-induced catalepsy and suggest that GABAergic and glutamatergic neurotransmission in the IC might be involved in paradoxical kinesia.

4.6.1 The inferior colliculus as a hub for paradoxical kinesia

The effect of NMDA agonist microinjected into the IC

In the study I playback presentation of an auditory 50-kHz USV stimulus reduced haloperidol-induced catalepsy in rats, representing an animal model to study paradoxical kinesia induced by an appetitive stimulus (Tonelli et al., 2018a). Furthermore, in study II, a first attempt was made to disclose the neural mechanisms involved in the paradoxical kinesia induced by species-specific ultrasounds. Regarding the role of the IC in the catalepsy induced by haloperidol, further evidence was obtained for an involvement of this structure since the glutamatergic agonist NMDA not only enhanced haloperidol-induced catalepsy, in line with previous results (Melo et al., 2010; Medeiros et al., 2014), but also prevented the effectiveness of the playback 50-kHz USV in inducing paradoxical kinesia in rats (Tonelli et al., 2018b). Significantly, the IC has projections to the deep and intermediate layers of the superior colliculus which is responsible for controlling head, eye and pinna movements for orientation toward sounds and objects in space (Casseday and Covey, 1996). Although the intracollicular microinjection of the glutamatergic agonist NMDA blocked the effect of 50-kHz USV, it had no effect on the basic auditory processing, i.e. rats showed pinna and head movements when exposed to all acoustic stimuli.

Remarkably, regarding the effects of intracollicular NMDA on 50-kHz USV playback, the IC serves not only as an important auditory relay structure including tonotopic representations for ultrasonic frequencies (Malmierca and Merchán, 2004), but also sends projections to pontine and medullary motor structures. In addition, the IC has outputs to the superior colliculus, and through that to the substantia nigra, thereby indirectly accessing the basal ganglia (Castellan-Baldan et al., 2006). Indeed, the IC is one of several brainstem sensorimotor structures which are not only indirectly connected to the basal ganglia via sensorimotor loops, but also to structures where basal ganglia outputs converge into a final common motor path to generate behavioral output (Redgrave et al., 2010; Olazábal and Moore, 1989; Moriizumi and Hattori, 1991). Therefore, even though DA transmission is impaired during neuroleptic-induced catalepsy (Sanberg, 1980), external auditory stimulation might induce paradoxical kinesia through the IC by activating motor circuits, a phenomenon which can be prevented by NMDA administration into the IC.

The effect of GABA agonist microinjected into the IC

In line with previous findings (Tostes et al., 2013), the GABA agonist diazepam microinjected into the IC potentiated the catalepsy induced by haloperidol in rats (Tonelli et al., 2018b). Remarkably, although intracollicular diazepam potentiated catalepsy in rats, 50-kHz USV playback was still able to induce paradoxical kinesia, i.e. rats were released from catalepsy. Notably, the playback of 50-kHz USV induced paradoxical kinesia in rats treated with the lower and the higher dose of diazepam, however, this effect was more prominent in the higher dose (Tonelli et al., 2018b). Moreover, as it was observed in response to intracollicular agonist NMDA, the basic auditory processing was not affected by the intracollicular microinjection of diazepam since the measures of pinna reflex and immediate head movements show that the rats perceived all the acoustic signals during the catalepsy test. Together, the present results suggest that the neurobiological mechanisms underlying paradoxical kinesia through the IC may be glutamatergic rather than GABAergic, since the NMDA agonist microinjected into the same structure suppressed the effectiveness of 50-kHz USV playback.

Overall, the IC mediates haloperidol-induced catalepsy via glutamatergic and GABAergic mechanisms. In the absence of an emotional or motivational auditory stimulus (50-kHz USV playback), both glutamatergic and GABAergic agonists microinjected into the IC potentiated haloperidol-induced catalepsy, probably by influencing descending auditory-motor pathways. Most recently, Takakusaki (2017) showed that the pedunculopontine nucleus (PPN) and the cuneiform nucleus (CnF), which form the neuroanatomical basis of the mesencephalic locomotor region (MLR), receive direct glutamatergic projections from the IC. In addition, glutamatergic PPN activity may facilitate slow, explorative locomotor behavior whereas those in the CnF promote escape locomotion (Caggiano et al., 2018). These brain structures might be involved in motor behavior driven by stimulation of the IC.

On the other hand, an auditory-amygdalar feedback may be recruited when rats are exposed to 50-kHz USV playback. In fact, there is a substantial and direct projection from the basal nucleus of the amygdala to the IC. These projections are distributed widely throughout the IC, including most of the central nucleus (ICC), i.e. the major recipient of ascending auditory brainstem input (Marsh et al., 2002). Although the role of these projections is unknown, the presence of such an auditory–amygdalar feedback circuit involving the IC may modify the processing of sound early in the ascending auditory pathway on the basis of an animal's emotional or motivational state. Possibly, this projection could explain these results

since glutamatergic, but not GABAergic, intracollicular mechanisms may be involved in this auditory-amygdalar feedback affecting the motor response (catalepsy) to a highly emotional and motivational auditory stimulus, i.e. 50-kHz USV playback. Ultimately, this auditory-amygdalar feedback may modulate the descending projection from the IC to the MLR to ensure an appropriate motor response.

4.7 Paradoxical kinesia and its possible mechanisms

There are basically three fundamental assumptions to mechanisms that might be involved in paradoxical kinesia; i.e. activation of basal ganglia reserves, activation of alternative pathways via cerebellar circuit or via noradrenergic augmentation. Therefore, these hypothetical approaches might correlate with current findings in this dissertation (Tonelli et al., 2018a; 2018b).

4.7.1 The basal ganglia

Essentially, the basal ganglia have a complex circuit which operates in order to promote normal motion ability. For instance, in PD, the degeneration of dopaminergic neurons in the SNc, which is one of the most principal nuclei of the basal ganglia, leads to motor impairments. The other nuclei of the basal ganglia are: the globus pallidus, the subthalamic nucleus and the striatum, which is anatomically divided into three subdivisions: the caudate nucleus, the putamen and the ventral striatum (including the nucleus accumbens). Furthermore, the basal ganglia have distinct pathways that are constantly competing with each other to produce movement or to inhibit it (Graybiel, 2000). The neurotransmitter DA is produced by the cells of the SNc whose axons project into the striatum; hence via direct or indirect pathways DA stimulates movement or inhibits it respectively.

Although there is a substantial decrease of DA release due to the degeneration of DA neurons in the SNc, PD patients are still able to produce DA deriving from different structures in the brain (Banou, 2015). There are several studies showing that the increase of DA release is associated with a reward process or even the expectation of a reward (De la Fuente-Fernandez, 2002; 2006). Moreover, studies have shown that the dorsal and ventral

striatum release DA when listening to pleasurable music, and activity in these structures also codes the reward value of musical excerpts (Zatorre, 2015). Auditory stimulation such as pleasurable music might increase the release of DA in patients with PD, helping them to override motor impairments (Salimpoor et al., 2011).

Most recently, Moraes et al. (2018), showed that auditory stimulation by exposure to melodic music increases DA activity in the forebrain areas linked with reward and motor control in rats. Interestingly, the appetitive playback of 50-kHz USV also increases DA release in the nucleus accumbens of naïve rats (Willuhn et al., 2014), which may underlie its approach-eliciting effects in intact animals and perhaps also in akinetic animals which have received haloperidol (Tonelli et al., 2018a). Overall, assuming that the basal ganglia play an important role in emotion-driven behavior and motor control (Zatorre et al., 2007), an emotional and motivational auditory stimulus might induce paradoxical kinesia by activation of DA reserves within the basal ganglia, or by routes by-passing them (Glickstein and Stein, 1991).

4.7.2 The cerebellar circuit

The cerebellar circuit might represent an interesting alternative pathway to explain paradoxical kinesia, especially because the cerebellum is responsible for preparation and execution of movement. For instance, it is known that in PD patients (OFF medication) there is hyperactivity in the cerebellum not only during motor activity but also at rest. Ballanger et al. (2006) mentioned that this hyperactivity in the cerebellum represents a compensatory process in order to reestablish normal motor movements. Importantly, the cerebellum, basal ganglia and structures of the midbrain are connected, integrating a functional network which might provide an anatomical basis for interpretation of the role of the cerebellum in PD. Furthermore, proper stimulation of these brain areas might interfere in the functionality and, hence, modulate motor responses. Speculatively, this dissertation provides evidence that the IC is involved in paradoxical kinesia and that this auditory structure sends direct glutamatergic projections to the pontine nuclei (ponto cerebellar auditory pathway; Saint Marie, 1996) which could be activating intact cerebellar circuits, thus providing an alternative motor response in cataleptic rats.

4.7.3 The noradrenergic system

In order to keep the survival instinct prompt, the noradrenergic system organizes the body's response with other body systems. Under life-threating events, "fight or flight" reactions are part of the response seen to adrenal activation by environmental stress (Yntema and Korf, 1987). Several reports demonstrated that akinetic PD patients exposed to life-threating events such as a fire, earthquake or the sound of a car accident were able to run out, and this may be due to noradrenergic activation (Yntema and Korf, 1987). Alongside this, Szot et al. (2011) argue that a deficiency in the noradrenergic system could worsen the progression of neurodegenerative diseases such as PD and Alzheimer.

Noradrenergic neurons release norepinephrine, also known as noradrenaline, a catecholamine activated in response to life-stress. Noradrenaline is presented in several brain functions, including learning and emotions (Avery et al., 2012). Interestingly, this might be linked with the fact that bradykinesia/akinesia is dependent on the emotional state of PD patients. In other words, an unexpected change of emotional energy in the brain can induce paradoxical kinesia and allow PD patients to activate intact motor programs which they are unable to do so without an external arousal (Jankovic, 2008). Moreover, another study about the involvement of the noradrenergic system in paradoxical kinesia observed that cataleptic rats, when exposed to stress (thrown into water), experienced paradoxical kinesia (Colpaert, 1987). Finally, a classical study presented robust evidence that life-stress factors induce paradoxical kinesia. Degryse and coworkers (1986), using the neurotoxin MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine), produced symptoms resembling those of Parkinson's, such as rigidity, tremor, bradykinesia and akinesia, in a monkey. Accordingly, once the monkey entered a complete state of akinesia, it was exposed to a natural enemy, the female German Shepherd. The monkey then got up, showing perfect motor control and started to run towards the dog in order to attack. As soon as the monkey was stopped by the examiner, it retreated to its previous postural situation. In conclusion, these reports could support the hypothesis that paradoxical kinesia might not be mediated by DA (Keefe et al., 1989).

4.8 Conclusions and Future Prospects

Undoubtedly, the understanding of how rats communicate via USV has reshaped the way behavior aspects are interpreted in neuroscience. Jaak Panksepp, who unfortunately died

last year, devoted most of his research career to reveal that the key to understanding human mental illness was the comprehension of primal emotional operating systems in conserved neural circuitry. Pankepp was a pioneer in the area of affective neuroscience who championed the use of experimental methods to study affective behavior in animals and the measurement of USV in rats to assess their affective state. Remarkably, since rats' USV represent the emotional state of the animals, they allow us to investigate diseases and disorders in which emotional aspects are valuable for the understanding of neural mechanisms.

Therefore, the scope of this dissertation was the development of a new animal model to investigate the phenomenon of paradoxical kinesia in rats using their own rats' USV as external auditory stimuli, as well as uncovering a possible neural mechanism involving the IC. Together, the present findings suggest that paradoxical kinesia can be induced in akinetic rats by the playback of natural appetitive 50-kHz USV and that this effect is regulated by glutamatergic but not GABAergic mechanisms in the IC. In this dissertation, the development of a new animal model of paradoxical kinesia provides a completely new approach to understanding how emotional components may be linked to paradoxical kinesia and substantially supports the pivotal role of the IC in this intriguing phenomenon. Namely, once the IC is stimulated by playback of 50-kHz USV, it might activate alternative motor circuits during paradoxical kinesia, restoring normal motor activity and hence overcoming the akinesia state induced by haloperidol in rats. Since, in this dissertation, an appetitive auditory stimulation was used, this new animal model of paradoxical kinesia presented might provide an excellent translational tool to clarify the neural mechanisms underlying the benefits of pleasurable auditory stimulation in PD patients such as music (Arias and Cudeiro, 2008; Rubinsten et al., 2002).

In summary, it is known that music is pleasant for PD patients and can sometimes produce the phenomenon of paradoxical kinesia. There are three particular aspects that are important in music for the patients: these are that the music is an auditory stimulus, it has emotional connotations and it has rhythm. The playback of 50-kHz USV presented to the rats in catalepsy also has two of these important aspects; the USV are an auditory stimulus which have emotional weight and meaning for the rats. In regard to the rhythm, although the playback of 50-kHz USV has a rhythm, it is hard to measure how relevant this rhythm is for this animal model of paradoxical kinesia since rats might not perceive rhythm in the same way that humans do. Nevertheless, this new animal model supports the use of pleasurable sounds as an emotional auditory stimulus for inducing paradoxical kinesia in rats.

In regard to future perspectives, these findings further extend the knowledge on paradoxical kinesia and open a new array of relevant scientific questions on which further investigations may focus. For instance, it would be interesting to consider looking at the features of playback of 50-kHz USV, particularly the types of 50-kHz calls that might be more relevant for rats during paradoxical kinesia. Another important aspect would be to investigate whether paradoxical kinesia could be produced by playback of 50-kHz USV in rats under influence of another type of drug that can also induce catalepsy, such as the opioid morphine, in which the dopaminergic system is not directly related. Lastly, one should consider looking into other brain structures which have direct projections from or to the IC and that might be involved in paradoxical kinesia, such as the amygdala (Marsh et al., 2002), the MGB (Winer et al., 1996), the SNpr (Olazábal and Moore, 1989), the globus pallidus (Moriizumi and Hattori, 1991) and the pontine nuclei (Saint Marie, 1996). Targeting these brain structures with microinjections for delivery of drugs, electrophysiology and optogenectic, are suitable techniques that can be applied to this new animal model of paradoxical kinesia presented in this dissertation.

5 PUBLICATIONS

Study I: Awakenings in rats by ultrasounds: A new animal model for paradoxical kinesia

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Research report

Awakenings in rats by ultrasounds: A new animal model for paradoxical kinesia

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Bar test

ABSTRACT

Paradoxical kinesia refers to a sudden transient ability of akinetic patients to perform motor tasks they are otherwise unable to perform. The mechanisms underlying this phenomenon are unknown due a paucity of valid animal models that faithfully reproduce paradoxical kinesia. Here, in a first experiment, we present a new method to study paradoxical kinesia by "awakening" cataleptic rats through presenting appetitive 50-kHz ultrasonic vocalizations (USV), which are typical for social situations with positive valence, like juvenile play or sexual encounters ("rat laughter"). Rats received systemic haloperidol to induce catalepsy, which was assessed by means of the bar test. During that test, 50-kHz USV, time- and amplitude-matched white noise (NOISE), or background noise (BACKGROUND) were played back and compared to SILENCE. Every animal was exposed to all four acoustic stimuli in random order, with four independent groups of rats being tested. Only when exposed to playback of appetitive 50-kHz USV, the otherwise akinetic rats rapidly started to move efficiently. The acoustic control stimuli, in contrast, did not release rats from catalepsy, despite eliciting the auditory pinna reflex and head movements towards the sound source. Moreover, in a second experiment, playback of aversive 22-kHz USV and relevant acoustic control stimuli did also not significantly affect catalepsy time. Together, our animal model provides a completely new approach to study mechanisms of paradoxical kinesia, which might help to improve behavioral therapies for Parkinson's disease and other disorders, where akinetic or cataleptic states occur.

1. Introduction

Bradykinesia (slowness of movement) and akinesia (loss of movement) is a state commonly characterized by inability or incapability to initiate a particular movement and a tendency to maintain an immobile posture, i.e. where the limbs remain in externally imposed positions [1]. Such states can occur in response to drug toxicity, high-dosed neuroleptics, such as haloperidol, or in neurological diseases, especially progressive lacunar cerebro-sclerosis, post-encephalitis and Parkinson's disease (PD) [2]. The latter is a neurodegenerative disease of the basal ganglia, especially its transmitter dopamine (DA), where bradykinesia and akinesia provide important clinical features. Interestingly, brady- and akinesia are dependent on the patient's emotional state [3]. For example, immobile patients excited by certain external stimuli may be able to make quick movements, such as catching a ball or running. There are several reports of this phenomenon called paradoxical kinesia, a term coined by Souques in 1921 [4] to describe "a sudden and brief period of mobility typically seen in response to emotional or physical stress" in patients with advanced PD. However, paradoxical kinesia is not restricted to stressful or even life-threatening events, since non-aversive visual and acoustic stimuli have also been shown to be effective. In fact, Oliver Sacks [5] was probably the first to note that familiar music can induce paradoxical kinesia, i.e. "temporary awakenings", in patients with severe akinesia. Since then, many clinical studies have shown that music or cueing sounds can be therapeutically valuable for PD patients to treat freezing of gait and akinesia [6,7].

The intriguing phenomenon of paradoxical kinesia has long been puzzling scientists, both in neurological and motor control research, with the neuronal mechanism still being a subject of debate. It is possible, for example, that PD patients have intact motor programs but

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have difficulty accessing them without external sensory stimulation [3,8] and paradoxical kinesia might work to improve motion by activation of basal ganglia reserves or via alternative pathways like cerebellar circuits to somehow improve motion [9]. Although these theories look convincing, their investigation is limited since paradoxical kinesia induced by life-threatening events, for example, cannot be investigated systematically in humans due to ethical constraints. In rats, catalepsy induced by systemic administration of haloperidol, which mainly acts by blocking DA D2 receptors [10,11], models the bradykinesia and lack of spontaneous motor activity that is common in some PD patients. However, the paucity of valid animal models to study paradoxical kinesia is impeding the discovery of the neural mechanism underlying paradoxical movements. Recently, we proposed that deep brain stimulation of the inferior colliculus (IC), a midbrain auditory structure, can provide an animal model to study paradoxical kinesia, in that case induced by aversive stimulation [12].

Considering that paradoxical kinesia can be observed not only after aversive but also appetitive stimulation, our present goal was to establish a completely new method for evaluating paradoxical kinesia after appetitive or aversive auditory stimulation in rats. Here, we assessed whether the presentation of an emotionally and motivationally relevant appetitive or aversive auditory stimulus may release rats from haloperidol-induced catalepsy, as measured by means of the bar test [13]. In a first experiment, we selected 50-kHz calls, i.e. rat ultrasonic vocalizations (USV), which are typical for social situations with positive valence, like juvenile play or sexual encounters [14,15], for auditory stimulation. These USV are thought to reflect a positive emotional state of the sender ("rat laughter") [16] and, in line with a pro-social communicative function, are known to induce behavioral activation and approach in the recipient [17]. Both effects are closely related to dopamine function in the nucleus accumbens [18,19]. In a second experiment, 22-kHz USV were selected for auditory stimulation, which are known as "alarm cries" [20] usually emitted in aversive situations, such as social defeat, predatory exposure, or fear conditioning [21,22].

2. Materials and methods

2.1. Animals

Male Wistar rats N = 68 (Charles River Deutschland), weighing between 200 and 250 g, were housed in polycarbonate cages (size: 380 × 200 × 590 mm) in groups of N = 4. Animals had *ad libitum* access to chow and water in a 12:12 dark/light cycle (lights on 07:00-19:00 h) and were allowed 10 days of acclimatization before testing. Then, each animal was handled on three consecutive days (5 min each day) and on the last day, it was brought to the testing room where it was habituated for 3 min to the observation arena. All experimental procedures were approved by the ethical committee of the local government (Regierungspräsidium Gießen, Germany, TVA Nr: 124-2014).

2.2. Experimental setup

An observation arena (100 cm^2), elevated 50 cm above the floor and containing 4 orifices with small home cages containing fresh bedding material beneath them, was used for testing. Cameras (Panasonic WV-BP330/GE, Hamburg, Germany) were placed above (~150 cm) and in front (~40 cm) of the arena. Acoustic stimuli were presented through an ultrasonic loudspeaker (ScanSpeak, Avisoft Bioacoustics, Berlin, Germany), using an external sound card with a sampling rate of 192 kHz (Fire Wire Audio Capture FA-101, Edirol, London, UK) and a portable ultrasonic power amplifier having a frequency range of 1–125 kHz (Avisoft Bioacoustics). The loudspeaker, which has a frequency range of 1–120 kHz with a relatively flat frequency response (± 12 dB) between 15 and 80 kHz, was placed 20 cm away from the observation arena. An additional, but inactive ultrasonic loudspeaker was arranged symmetrically at the opposite side as visual control. Playback of acoustic stimuli was monitored by an UltraSoundGate Condenser Microphone (CM16; Avisoft Bioacoustics) placed next to the speaker.

2.3. Catalepsy test

Catalepsy was induced by injecting haloperidol (0.5 mg/kg) intraperitoneally (IP) 60 min before placing the rat onto the observation arena. Rats were individually brought from their home cage to the testing room 15 min before haloperidol injection and placed into a single cage containing bedding material. The subsequent bar test [13] consisted of gently placing the rat with its forepaws on a horizontal bar positioned 8 cm above the floor of the arena. The time until it stepped down with both forepaws was measured (maximum 300s). Acoustic stimuli were presented for 270 s (total test duration: 30s + 270s = 300s), followed by an inter-stimulus interval of 300s. Rats were tested in a room with no experimenter or other rats present. Stimulus application and animal observation were performed between 8 and 18 h under dim red light (~10 lx). Before each test, behavioral equipment was cleaned (0.1% acetic acid solution) and dried.

2.4. Experiment design

2.4.1. Experiment 1: effects of 50-kHz USV on haloperidol-induced catalepsy

To test the effects of 50-kHz USV in cataleptic animals (N = 48), the bar test was performed during which a given rat was exposed to different playback presentations of (i) 50-kHz USV, (ii) time- and amplitude-matched white noise (NOISE), (iii) background noise (BACKGROUND) and (iv) SILENCE (for experimental setup and exemplary spectrograms, see Fig. 1a–d). Every animal was exposed to all four acoustic stimuli in random order, at 60, 70, 80 and 90 min after injection of haloperidol, resulting in four independent groups i.e. in N = 12 rats, the first acoustic stimulus was 50-kHz USV, NOISE, BACKGROUND or SILENCE, respectively. Playback was started after the rat remained for at least 30 s in catalepsy, i.e. with its forepaws on the horizontal bar; N = 5 rats were excluded from analysis because they did not fulfill this criterion.

2.4.2. Experiment 2: effects of 22-kHz USV on haloperidol-induced catalepsy

To test the effects of 22-kHz USV in cataleptic animals (N = 20), the bar test was performed during which a given rat was exposed to different playback presentations of (i) 22-kHz USV, (ii) phase-scrambled and frequency-shifted 22-kHz USV (22-kHz USV CONTROL) and (iii) SILENCE. Since it is assumed that the motivational impact of 22-kHz USV may depend on experience, namely to perceive them as aversive [22,23], half of the rats underwent an autoconditioning procedure (adapted from Parsana et al., see [24]), before the playback test.

To this aim, rats were divided into two groups (autoconditioned and non-autoconditioned), which were otherwise treated and housed under the same conditions as described above. Rats were autoconditioned using a footshock procedure starting three days before being exposed to playback of 22-kHz USV. This procedure consisted of placing a rat in a footshock chamber (dimensions of 33.5 cm wide × 35 cm deep × 38 cm high) with a stainless-steel grid for five minutes (day 1). On that day, no footshocks were applied. The next day (day 2), the rat was again placed into the chamber, being exposed to a 180 s baseline period followed by unsignaled footschocks (three to five shocks were delivered; 0.8 mA, 0.5s) with an inter-trial interval of 180 ± 21s. In case, rats emitted 22-kHz USV before or soon after the third shock no more footshocks were delivered. The other group underwent the same procedure except that footshocks were not applied. All the rats remained a total of 18 min inside the chamber. The hypothesis predicts that the behavioral responsiveness to 22-kHz USV playback is related to emitting 22-kHz USV during the aversive experience, in our case nine

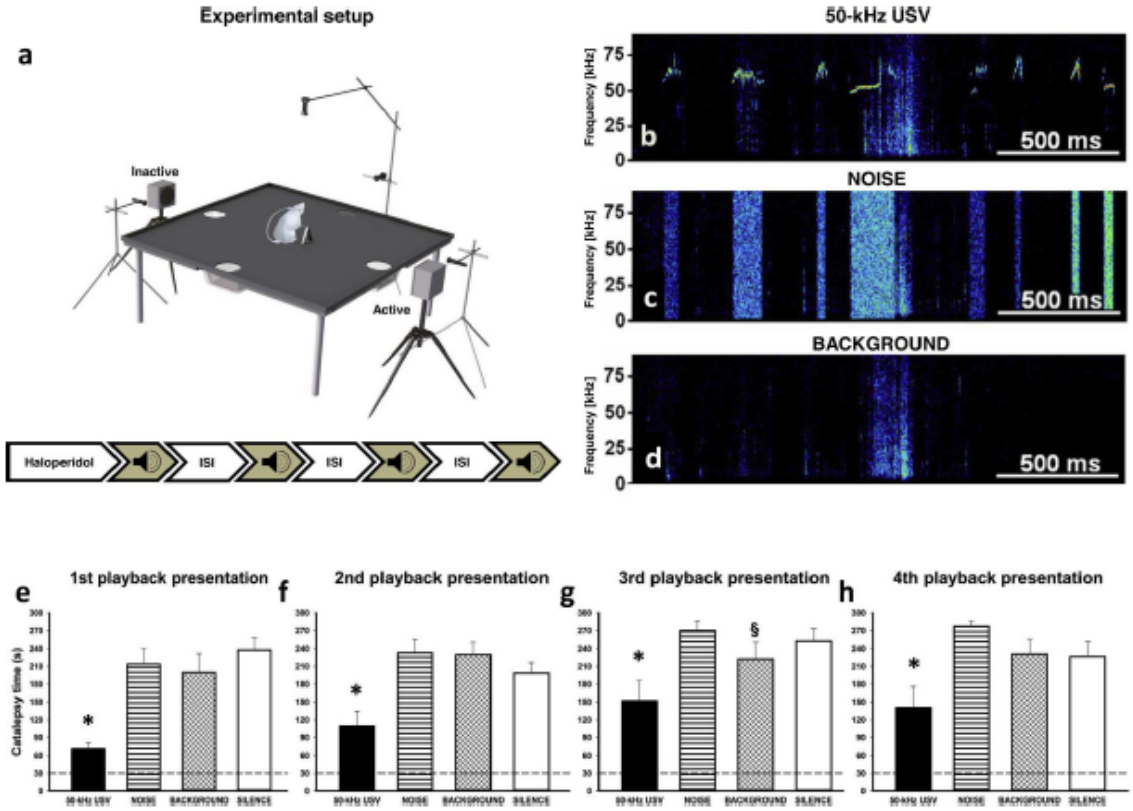


Fig. 1. Experimental setup and effects of 50-kHz USV on haloperidol-induced catalepsy. (**a**) Top view of the experimental setup and time line. (**b–d**) Exemplary spectrograms of 50-kHz USV, time-and amplitude-matched white noise (NOISE), and background noise (BACKGROUND) used for playback. (**e–h**) Catalepsy times (i.e. durations until rats stepped down from the bar) under haloperidol-induced catalepsy and playback of different auditory stimuli. Bars represent means + SEM during 50-kHz USV, NOISE, BACKGROUND and SILENCE for the 1st, 2nd, 3rd and 4th playback presentation. * $P < 0.05$, as compared with NOISE, BACKGROUND and SILENCE, except 50-kHz USV compared with BACKGROUND during the 3rd playback presentation (§ $P = 0.054$). Dashed line represents the criterion of 30 s to start playback presentation.

out ten animals vocalized throughout the autoconditioning. On the following day (day 3), rats were kept in their home cages and no experiment was performed. On the day thereafter, rats were taken to the catalepsy test where they were exposed to playback (adapted from Parsana et al., see [24]). There, a given rat was exposed to different playback presentations of (i) 22-kHz USV, (ii) 22-kHz USV CONTROL and (iii) SILENCE. Every animal was exposed to all three acoustic stimuli in random order, at 60, 70 and 80 min after injection of haloperidol. Playback was started after the rat had remained for at least 30 s in catalepsy, i.e. with its forepaws on the horizontal bar.

2.5. Acoustic stimuli

Rats in the first experiment were exposed to playback of (i) 50-kHz USV, (ii) time- and amplitude-matched white noise (NOISE), and (iii) background noise (BACKGROUND), but also (iv) SILENCE. NOISE and BACKGROUND were presented to control for unspecific effects not linked to the socio-affective communicative function of 50-kHz USV. In the second experiment rats were exposed to playback of (i) 22-kHz USV, (ii) phase-scrambled and frequency-shifted 22-kHz USV (22-kHz USV CONTROL) and (iii) SILENCE. All stimuli were presented with a sampling rate of 192 kHz in 16 bit format at ~69 dB (measured from a distance of 40 cm), with the exception of BACKGROUND, which was presented at ~50 dB, i.e. corresponding to the intensity of background noise present in the other acoustic stimuli.

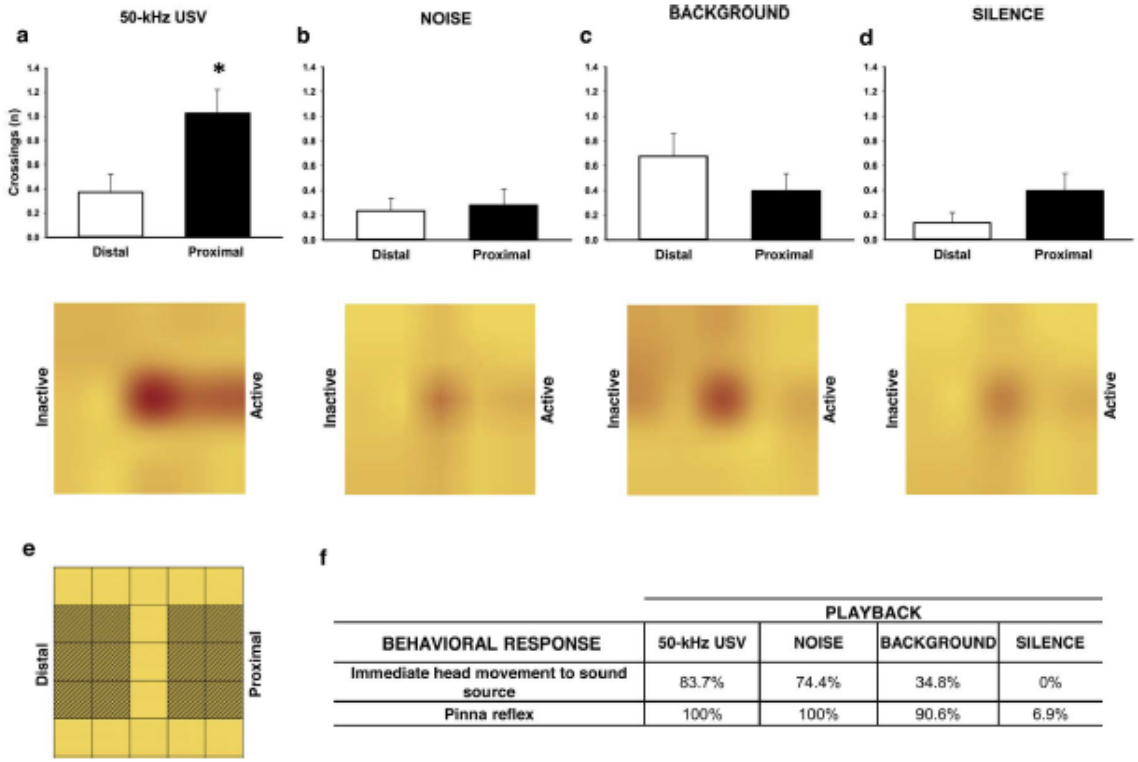
A 50-kHz USV: The 50-kHz USV had been recorded from an adult male Wistar rat during exploration of a cage containing scents from a cage mate after being separated from it (for setting and recording, see 19). The acoustic stimulus material was composed of a sequence lasting 3.5s, which was presented in a loop. Each sequence contains 13 50-kHz calls (total calling time: 0.90s), with 10 of them being frequency-modulated and 3 flat.

B NOISE: The artificial time- and amplitude-matched white noise was generated with SASLab Pro (Version 4.2, Avisoft Bioacoustics). Specifically, each given 50-kHz USV in the original natural 50-kHz USV stimulus material was replaced by white noise with durations and amplitude modulations identical to those of the original 50-kHz USV. Thus, the stimulus series had the same temporal patterning and was identical to the original natural 50-kHz USV series with respect to all call features, apart from the fact that sound energy was not confined to a certain frequency as in case of the natural 50-kHz USV.

C BACKGROUND: Since the 50-kHz USV stimulus contained background noise, i.e. noises, which occur when a rat is exploring an arena with bedding, background noise without 50-kHz USV was presented.

D 22-kHz USV: The 22-kHz USV were recorded from a male Wistar rat which had received electric footshocks before, but not during recording (for setting and recording, see 19). The acoustic stimulus contained 29 22-kHz USV per min. Average acoustic call parameters were as follows (mean ± SEM): call duration: 1.18 ± 0.06s; peak frequency: 23.61 ± 0.07 kHz; frequency modulation: 1.90 ± 0.09 kHz. To control for background noise present in the original natural 50-kHz USV stimulus, background noise was added to 22-kHz USV stimulus.

E 22-kHz USV CONTROL: The phase-scrambled and frequency-shifted 22-kHz USV control was generated with SASLab Pro (Version 4.2, Avisoft Bioacoustics). Specifically, each given 22-kHz USV in the original natural 22-kHz USV stimulus material was first phase-



	PLAYBACK			
BEHAVIORAL RESPONSE	50-kHz USV	NOISE	BACKGROUND	SILENCE
Immediate head movement to sound source	83.7%	74.4%	34.8%	0%
Pinna reflex	100%	100%	90.6%	6.9%

Fig. 2. Effects of 50-kHz USV on exploratory behavior. (**a–d**) Exploratory behavior displayed by rats that stepped down from the bar when exposed to playback of 50-kHz USV (N = 29), NOISE (N = 8), BACKGROUND (N = 21), and SILENCE (N = 13), as measured by counting quadrant crossings in the zone distal from and proximal to the active ultrasonic speaker, respectively. Heat maps depict corresponding spatial distributions of exploratory behavior. (**e**) Hatched areas indicate proximal and a distal zone, i.e. one close to and the other opposite from the sound source, respectively. Each zone (24% of the arena) was divided into 6 quadrants. Data are expressed as means + SEM. * $P < 0.05$, as compared with the distal zone. (**f**) Percentage of rats displaying pinna reflex and immediate head movements towards the sound source.

scrambled, i.e. the phase of the original signal was replaced by a random phase. The resulting signal exhibiting the original average power spectrum, but its waveform being a random noise signal, was then shifted up in frequency by 25 kHz.

F SILENCE: Rats were not exposed to any acoustic stimulus during this block of testing, i.e. they were submitted to the catalepsy test only.

2.6. Behavioral analysis

In order to verify that all acoustic stimuli were audible to the rats, in addition to catalepsy time, we assessed two behaviors while the rat remained with its forepaws on the bar during playback: (i) pinna reflex, a rapid and intermittent pinna movement, used to study the integrity of the lower auditory pathway [25];and (ii) immediate head movement toward the sound source. The number of rats displaying these behaviors was counted. In cases where the animals stepped down from the bar and started to explore the arena, we quantified this behavior by dividing the arena into a proximal and a distal zone, i.e. one close to and the other opposite from the sound source, respectively, and each zone (24% of the arena) was further divided into 6 quadrants (see Fig. 2e). Exploratory behavior was then assessed by counting quadrant crossings, defined as when the rat crossed a quadrant with its four paws.

2.7. Statistical analysis

In the first experiment, catalepsy time was analyzed using ANOVAs, followed by Student-Newman-Keuls post-hoc test when appropriate. Groups were compared at minutes 60–65 (1st playback), 70–75 (2nd playback), 80–85 (3rd playback), and 90–95 (4th playback) after haloperidol injection. In addition, a paired-sample *t*-test (2-tailed) was used to compare exploratory behavior between the proximal and distal zone. In the second experiment, catalepsy time was again analyzed using ANOVAs. Groups were compared at minutes 60–65 (1st playback), 70–75 (2nd playback) and 80–85 (3rd playback) after haloperidol injection. In both experiments, Chi^2 tests were used to analyze the occurrence of pinna reflex and immediate head movement. Statistical analyses were performed using IBM SPSS software Statistics 22. A *p*-value of < 0.05 was considered statistically significant.

3. Results

3.1. Experiment 1: effects of 50-kHz USV on haloperidol-induced catalepsy

When the cataleptic rats received playback of 50-kHz USV, they rapidly started to move and typically stepped down from the bar (for experimental setup and exemplary spectrograms, see Fig. 1a–d). Intriguingly, most of them even walked towards the active ultrasonic speaker. This effect was specifically seen in response to appetitive 50-kHz USV and not in response to various acoustic control stimuli used to test for unspecific effects not linked to the socio-affective communicative function of 50-kHz USV. Thus, when quantifying catalepsy time, only 50-kHz USV playback substantially reduced step-down latencies. Importantly, this effect was consistently observed in four independent groups of rats and irrespective of whether 50-kHz USV were presented during the 1st ($F_{3,39} = 11.88$, $P < 0.001$), 2nd ($F_{3,39} = 7.22$, $P = 0.001$), 3rd ($F_{3,39} = 4.42$, $P = 0.009$) or 4th ($F_{3,39} = 4.76$, $P = 0.006$) playback presentation (Fig. 1e–h), with the effects being most prominent during the 1st presentation. In contrast, playback of acoustic control stimuli, i.e. NOISE and BACKGROUND, did not significantly affect catalepsy time (*P*-values > 0.05). In fact, catalepsy time during NOISE and BACKGROUND was comparable to SILENCE, suggesting that, out of the acoustic stimuli applied here, only playback of 50-kHz USV was efficient to release rats from haloperidol-induced catalepsy.

In addition, we analyzed the behavior of the rats, which stepped down from the bar in more detail. After stepping down, rats exposed to 50-kHz USV explored the zone proximal to the active ultrasonic speaker more than the distal one ($t = 2.375$, $P = 0.022$; N = 29 cases; Fig. 2a). In contrast, the rats that managed to step down from the bar during NOISE, BACKGROUND, or SILENCE (N = 8, N = 21, and N = 13; respectively) did not exhibit exploratory behavior or any preference for the sound source (all *P*-values > 0.050; Fig. 2b–d). This further supports the notion that it is only when exposed to the 50-kHz USV playback that rats are released from catalepsy and able to move in the direction of the sound source, i.e. clearly displaying approach behavior (see Supplementry videos 1 and 2). Remarkably, soon after the 50-kHz USV presentation ended, the rats displayed catalepsy again.

The fact that rats were released from catalepsy specifically in response to 50-kHz USV was not due to an inability to hear the control stimuli. The pinna reflex was detected in most cases of our acoustic stimuli (50-kHz: 43 out of 43; NOISE: 43/43, BACKGROUND: 39/43), but not during silence (3/43). Also, we observed head movements towards the sound source as soon as the acoustic stimulus started, with 36/43, 32/43, and 15/43 rats responding to 50-kHz USV, NOISE, and BACKGROUND, respectively. No such head movements were seen during SILENCE (Fig. 2f). Together, this clearly shows that all three acoustic stimuli were perceived by the rats (all *P*-values < 0.001 compared with SILENCE).

3.2. Experiment 2: effects of 22-kHz USV on haloperidol-induced catalepsy

In contrast to 50-kHz USV, playback of 22-kHz USV did not significantly alter catalepsy time. Neither, the group which had undergone prior autoconditioning ($F_{2,29} = 0.029$, $P > 0.050$; 22-kHz USV: 220.40 ± 29.23s; 22-kHz USV CONTROL: 208.60 ± 30.07s; SILENCE: 210.60 ± 32.40s) nor the non-autoconditioned one ($F_{2,29} = 0.049$, $P > 0.050$; 22-kHz USV: 200.40 ± 33.70s; 22-kHz USV CONTROL: 188.50 ± 32.34s; SILENCE: 188.00 ± 27.20s) were affected by the auditory stimuli. Although these rats did not step down from the bar when exposed to the 22-kHz signals, most of them, while on the bar, showed pinna reflex and head movement towards the sound source, implying that they were perceiving the auditory stimuli (results not shown in detail).

4. Discussion

To the best of our knowledge, this is the first time that appetitive 50-kHz USV has been used as an external trigger to induce paradoxical kinesia in rats. Previous reports showing improved haloperidol-induced catalepsy in response to acoustic stimuli required either substantial prior training [26] or used spectrographically undefined sounds (like key jingles) and simple righting responses to restore balance [8]. Our present findings were based on spectrographically well-characterized and ethologically valid signals, and rather complex approach responses, including orienting, stepping down, and coordinated locomotion towards the active ultrasonic speaker. Furthermore, we show for the first time that these effects require specific acoustic features, since 50-kHz call sequences but not time- and amplitude-matched white noise were effective in reversing catalepsy, indicating that mere arousal is not sufficient for this outcome. This parallels our previous results in undrugged rats, where we showed that playback of such 50-kHz USV, but not various control stimuli, induces locomotion and approach [17], which highlights their motivational relevance as social signals. Interestingly, familiarity with a given sound [8] or its meaningfulness [27] seem to enhance the anti-cataleptic properties of acoustic stimuli in humans, which may be relevant here, since our ultrasonic signals are part of the rats' communicative repertoire [14,15], i.e. they fulfill the requirements of familiarity and meaningfulness. Also, it has been suggested that external stimuli lead to paradoxical kinesia by "energizing" relevant action systems in the brain [28], which are otherwise insufficiently activated. Here, 50-kHz calls may be especially suitable, since they have motivational properties, i.e. approach-inducing quality for the recipient [17].

Catalepsy time during NOISE and BACKGROUND was comparable to SILENCE, suggesting that, out of the acoustic stimuli applied here, only playback of 50-kHz USV was efficient to release rats from haloperidol-induced catalepsy. In addition, after stepping down, only rats exposed to 50-kHz USV explored the zone proximal to the active ultrasonic speaker more than the distal one. This further supports the notion that it is only when exposed to the 50-kHz USV playback that rats are released from catalepsy and able to display approach behavior (see Supplementary videos 1 and 2). Interestingly, soon after the 50-kHz USV presentation ended, the rats displayed catalepsy again. This is reminiscent of PD patients, who revert to akinesia after an auditory or visual stimulus has induced paradoxical kinesia, strongly supporting the face validity of our animal model. The fact that rats were released from catalepsy specifically in response to 50-kHz USV was not due to an inability to hear the control stimuli. Pinna reflex and head movements towards the sound source were detected in most cases of our acoustic stimuli but not during SILENCE, showing that all three acoustic stimuli were perceived by the rats.

In contrast to 50-kHz USV, playback of 22-kHz USV was not effective to reduce haloperidol-induced catalepsy, even though we tried to enhance their motivational properties by prior autoconditioning [24] in half of the subjects. At first sight, this result might indicate that these aversive acoustic stimuli are no effective in the cataleptic state, which is in contrast to classical clinical findings in PD patients [29,30] and to our previous experiments with aversive deep brain stimulation of the inferior colliculus (IC), a midbrain auditory structure being important for processing acoustic messages and mediating aversive states [12]. Regarding our present findings, however, one has to consider that the typical response to playback of 22-kHz USV in most rat strains is reduced activity or transient immobility [22], including Wistar rats used here [17], which might not be compatible with the current model of testing haloperidol-induced catalepsy. Therefore, it cannot be excluded that 22-kHz USV might be effective to reduce haloperidol-induced catalepsy. One way to further test this question could be to establish an active flight response to 22-kHz USV before testing their effects in the cataleptic state.

Despite these possible limitations regarding 22-kHz USV, our animal model of paradoxical kinesia in response to 50-kHz USV has several advantages over existing ones. First of all, familiar and meaningful ethologically valid signals are applied, which are precisely defined, and between-subject variance can easily be minimized due to the present playback approach, with all subjects being exposed to the exact same stimulus. Secondly, no training is required since the response elicited by 50-kHz USV is an unconditioned one. Thirdly, our new paradigm allows the study of paradoxical kinesia without exposure to aversive stimuli [8,31], which might allow to study how pleasant and appetitive stimuli exert their promotive effects in akinetic or cataleptic human subjects. Finally, the fact that rats are released from catalepsy in response to an emotionally and motivationally relevant appetitive auditory stimulus, but become cataleptic again immediately after it is turned off, mimics findings on paradoxical kinesia in humans, and thus supports the model's face validity.

It is known that haloperidol-induced catalepsy is largely due to blockade of DA D2 receptors in the striatum, where they are located on GABAergic projection neurons and cholinergic interneurons [31,32] modeling the inability of PD patients to initiate movements and thus reproducing key DAergic aspects of PD. Paradoxical kinesia is generally thought to be mediated either by reserves within the basal ganglia, or by routes by-passing them [9]. With respect to the former, it is important to note that playback of 50-kHz USV leads to phasic DA release in the nucleus accumbens [19], which may underlie their approach-eliciting effects in intact animals. It remains to be shown whether such effects are also possible in case of rather complete blockade of D2

receptors as used here, especially since it is thought that paradoxical kinesia is not mediated by actions on striatal DA [33].

With respect to alternative routes, previous studies conducted in our laboratory have shown that both systemic and intrastriatal haloperidol-induced catalepsy can be significantly reduced by prior microinjection of the NMDA glutamate receptor antagonist MK-801 into the IC [34,35]. We also demonstrated that microinjection of bicuculline, a GABAergic antagonist, directly into the IC induced a biphasic effect progressing from attenuation to potentiation of catalepsy induced by systemic haloperidol [36]. Furthermore, we had recently shown that high frequency deep brain stimulation of the IC reduced haloperidol-induced catalepsy [12], representing an animal model of paradoxical kinesia induced by aversive stimulation, since these stimulation parameters led to flight reactions. Moreover, regarding the mechanisms possibly underlying paradoxical kinesia, previous studies from our laboratory strongly suggest that the auditory midbrain plays a critical role. External auditory stimulation activating the inferior colliculus may trigger motor circuits even when striatal dopamine transmission is impaired during neuroleptic-induced catalepsy [34,35].

Together, we propose a new animal model to investigate paradoxical kinesia in rats, which can contribute to clarify the psychological and neural mechanism underlying this intriguing phenomenon. Uncovering these mechanisms might help to improve current and develop new noninvasive therapies for PD and other disorders, where akinetic or cataleptic states occur.

Author's roles

L.C.T. performed the behavioral experiments and data analysis. M.W. developed the playback methodology. L.M.T. conceived the study, developed the methodology, acquired funding, and approved the final version to be published. All authors actively participated in designing the experiment, interpreting all data, and editing the manuscript.

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Relevant conflicts of interests/financial disclosures

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2017.09.021.

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Study II: Paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats depends on glutamatergic mechanisms in the inferior colliculus

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Paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats depends on glutamatergic mechanisms in the inferior colliculus

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ABSTRACT

Paradoxical kinesia is a sudden transient ability of akinetic patients to perform motor tasks they are otherwise unable to perform. This phenomenon is known to depend on the patient's emotional state and external stimuli. Paradoxical kinesia can be induced by appetitive 50-kHz ultrasonic vocalizations (USV) in rats displaying catalepsy following systemic haloperidol. We investigated the role of the inferior colliculus (IC) in paradoxical kinesia induced by 50-kHz USV, since the IC modulates haloperidol-induced catalepsy. We focused on glutamatergic and GABAergic neurotransmission, with male rats receiving intracollicular NMDA or the GABA receptor agonist diazepam 10 min before systemic haloperidol. Catalepsy time was assessed by means of the bar test, during which rats were exposed to playback of 50-kHz USV, white noise, and background noise. Our results show that playback of 50-kHz USV induced paradoxical kinesia by reducing haloperidol-induced catalepsy in rats which had received saline intracollicular microinjection. This paradoxical kinesia effect of 50-kHz USV playback on haloperidol-induced catalepsy was prevented by intracollicular NMDA administration. Although intracollicular diazepam microinjection potentiated haloperidol-induced catalepsy, it did not affect the response to 50-kHz USV playback. Together, NMDA receptor agonist suppressed the effectiveness of 50-kHz USV playback, whereas diazepam did not. These findings suggest that the IC is a key structure involved in paradoxical kinesia, with relevant processes being glutamatergic rather than GABAergic. Our approach thus appears useful for uncovering neural mechanisms of paradoxical kinesia and it might help identifying novel therapeutic targets for Parkinson's disease.

1. Introduction

Patients with Parkinson's disease (PD), a neurodegenerative basal ganglia disease, present a global deterioration in motor function with bradykinesia – slowness of movement – as one of the most characteristic clinical features, which, in extreme cases, can lead to an almost complete loss of movement, termed akinesia (Schilder et al., 2017). Such states can also occur in response to drug toxicity, high-dosed neuroleptics, such as haloperidol, or in other neurological diseases, especially multiple system atrophy (Wenning et al., 2004), progressive lacunar cerebro-sclerosis, or post-encephalitis (Schilder et al., 2017; Satterthwaite et al., 2008). It is known that akinesia depends on the emotional state of the subject and certain external stimuli (Jankovic, 2008). For instance, akinetic parkinsonian patients, when properly stimulated by visual or auditory stimuli, can be able to perform tasks, such as catching a ball, riding a bicycle or running, which they were otherwise unable to perform. This intriguing phenomenon, called paradoxical kinesia was first named by Souques in 1921 (Souques, 1921) to describe "a sudden and brief period of mobility typically seen in response to emotional or physical stress" in patients with advanced PD. Interestingly, paradoxical kinesia is not restricted to stressful or even life-threatening events, since familiar music can also induce

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paradoxical kinesia in patients (Sacks, 1973; Rubinsten et al., 2002). Clinical application of this intriguing phenomenon is somehow limited, especially since the neural mechanisms underlying it are largely unknown in humans. Therefore, animal models are required and akinesia is commonly studied in rodents in terms of catalepsy, that is, a state of immobility in which the animals are unable to correct externally imposed postures. Such a state can be induced in rats, for example, by systemic or intrastriatal administration of haloperidol which mainly acts by blocking striatal post-synaptic dopamine (DA) D2 receptors (Hornykiewicz, 1973; Wadenberg et al., 2001), and this state mimics the lack of spontaneous motor activity that is commonly seen in some PD patients.

Using the haloperidol-induced catalepsy model in rats, we recently showed for the first time that playback of 50-kHz ultrasonic vocalizations (USV) can be used as an emotionally and motivationally relevant appetitive auditory stimulus to reproduce paradoxical kinesia in cataleptic rats (Tonelli et al., 2018). In general, USV are a prominent component of the behavioral repertoire displayed by rats and serve important communicative functions as situation-dependent socio-affective signals (Brudzynski, 2013; Wöhr and Schwarting, 2013). Specifically, 50-kHz USV are typical for social situations with positive valence, like juvenile play (Knutson et al., 1988) or sexual encounters (Barfield and Geyer, 1972), and are believed to reflect a positive affective state ("rat laughter"; Panksepp, 2005). As repeatedly shown by means of our 50-kHz USV radial arm maze playback paradigm, appetitive 50-kHz USV lead to social approach behavior in the recipient (Wöhr and Schwarting, 2007; Engelhardt et al., 2017). At the neurobiological level, this is accompanied by reduced neural activity in the amygdala (Parsana et al., 2012) but enhanced DA activity in the nucleus accumbens (Willuhn et al., 2014), a brain area implicated in reward processing (Choi and Brown, 2003).

On the other hand, it is known, that catalepsy in rats can be modulated by glutamatergic and GABAergic mechanisms in the inferior colliculus (IC; Melo et al., 2010; Tostes et al., 2013), a midbrain structure not only implicated in auditory processing, but also motor outputs, probably mediated by its connections with motor systems (Casseday and Covey, 1996). The IC is a relay station integrating descending and ascending auditory information; the latter is known as the main auditory thalamic relay, projecting from the IC to the medial geniculate body and thus to the auditory cortex (Cappe et al., 2009). Previously, we have shown that intracollicular microinjections of glutamatergic drugs can modulate haloperidol-induced catalepsy. Specifically, administration of the NMDA glutamate receptor antagonist MK-801 into the IC significantly reduced catalepsy time, whereas the agonist NMDA potentiated it (Melo et al., 2010). In addition, we showed that intracollicular microinjection of the GABAergic agonist midazolam potentiated haloperidol-induced catalepsy whereas the GABAergic antagonist bicuculline produced a biphasic effect (Tostes et al., 2013). Finally, we obtained evidence indicating that catalepsy induced by haloperidol can be reduced by high frequency electrical deep brain stimulation in the IC, representing an animal model of paradoxical kinesia induced by aversive stimulation, since this stimulation led to flight responses (Melo-Thomas and Thomas, 2015).

Together, the IC represents a prime candidate target to investigate paradoxical kinesia mechanisms induced by appetitive 50-kHz USV, since it not only relays auditory information (Casseday and Covey, 1996), but also modulates catalepsy. Here, we addressed the question whether the IC is involved in paradoxical kinesia induced by appetitive 50-kHz USV and whether such 50-kHz USV might become ineffective in counteracting haloperidol-induced catalepsy once microinjections of the glutamate agonist NMDA or the GABAergic agonist diazepam are delivered into the IC.

2. Material and methods

2.1. Subjects

N = 44 male Wistar rats (Charles River Deutschland), weighing between 200 and 250 g, were used. They had 7 days of acclimatization before surgery during which they were kept in groups (maximum of five animals per cage). All experimental procedures were approved by the ethical committee of the local government (Regierungspräsidium Gießen, Germany, TVA Nr: 124–2014).

2.2. General overview

Two experiments were performed, each consisting of two parts. In Experiment 1, we focused on glutamatergic neurotransmission and delivered the glutamate agonist NMDA into the IC through microinjection. In the first part of Experiment 1, we determined the effects of NMDA on haloperidol-induced catalepsy. In the second part, the effects of NMDA on paradoxical kinesia induced by 50-kHz USV playback were assessed, and compared to acoustic control stimuli. In Experiment 2, we focused on GABAergic neurotransmission and delivered the GABA agonist diazepam into the IC. Again, we first determined the effects of GABA on haloperidol-induced catalepsy without 50-kHz USV playback. Then, in the second part, the effects of GABA on paradoxical kinesia induced by 50-kHz USV playback were assessed.

2.3. Surgery

The animals were anesthetized with isoflurane (Baxter Deutschland GmbH, Germany) and mounted in a stereotaxic frame (TSE Systems, Bad Homburg, Germany). The upper incisor bar was set at 3.3 mm below the interaural line so that the skull was horizontal between bregma and lambda. Each animal was implanted unilaterally with guide cannulae (gauge 22, length 13 mm; Thomas Recording GmbH, Gießen, Germany) aimed at the IC using the following coordinates, with lambda serving as reference: anteroposterior = −1.2 mm; mediolateral = +1.5 mm; and dorsoventral = 4.5 mm (Paxinos and Watson, 2007). The guide cannulae were fixed to the skull with acrylic resin and three stainless steel screws. A stylette inside the guide cannula prevented obstruction. All animals were allowed a recovery period of 7 days after surgery with *ad libitum* access to food and water. During this period, they were kept in pairs.

2.4. Drug and doses

Haloperidol (Janssen Pharmaceutica, Beerse, Belgium) was obtained in a commercial form for intravenous use, in which the drug is dissolved in 1 ml of vehicle solution containing 6 mg lactic acid diluted with physiological saline to obtain the required concentration of 1 mg/ml. In Experiment 1, N-methyl-D-aspartic acid (NMDA; Sigma-Aldrich, Darmstadt, Germany) was dissolved in physiological saline, obtaining a dose of 30nmol/0.5 µl, which had previously been shown to increase haloperidol-induced catalepsy (Melo et al., 2010). In Experiment 2, 7-Chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepin-2(1H)-one (Diazepam; Sigma-Aldrich, Darmstadt, Germany) was dissolved in propylene glycol (10%), obtaining doses of 10µg/0.5 µl and 20µg/0.5 µl.

2.5. Microinjection procedure

A given microinjection was delivered using a 30 gauge stainless steel cannula introduced through the guide cannula until its lower end was 1 mm below the cannula tip. This infusion cannula was

connected to a 10 µl Hamilton syringe by polyethylene tube, and a volume of 0.5 µl of drug solution was delivered over 1 min. The animals from the control groups received an equivalent volume of physiological saline or vehicle. The cannula was left in place for an additional 1 min thereafter.

2.6. Induction and evaluation of catalepsy

First, the effects of intracollicular NMDA and GABA on haloperidol-induced catalepsy were assessed. To this aim, each animal was handled on three consecutive days (5 min each day) and on the last day it was brought to the testing room and habituated for 3 min to the observation arena prior to drug testing. Subjects either received intracollicular administration of NMDA (30nmol/0.5 µl), diazepam (10µg/0.5 µl or 20µg/0.5 µl) or its respective controls (i.e. physiological saline or vehicle) 10 min before haloperidol (0.5 mg/kg; ip). The catalepsy time was measured during the bar test, which consists of gently placing the rat with its forepaws on a horizontal bar positioned 8 cm above the floor of the arena. The time until it stepped down with both forepaws was measured (maximum 600s). The bar test was performed at 20, 40, 60, 80, 100 min after intracollicular administration.

2.7. Catalepsy test and playback

After a washout period of 72 h, the effects of intracollicular NMDA and GABA on paradoxical kinesia induced by 50-kHz USV playback were assessed, and compared to acoustic control stimuli. To this aim, the same groups of animals received either haloperidol (0.5 mg/kg; ip) 10 min after intracollicular administration of NMDA (30nmol/0.5 µl) or diazepam (10µg/0.5 µl or 20µg/0.5 µl), physiological saline or vehicle. Approximately 50 min later, catalepsy was verified by assuring that rats remained on the bar at least 2 min during silence. All animals fulfilled this criterion. Then, the rats were placed again on the bar and after 30s they were exposed to playback of 50-kHz USV, time- and amplitude-matched white noise (NOISE) and background noise (BACKGROUND), 10 min each with 5 min intervals.

2.8. Experimental setup

An observation arena (100 cm^2), elevated 50 cm above the floor and containing 4 orifices with small home cages containing fresh bedding material beneath them, was used for testing. Two cameras (Panasonic WVBP330/GE, Hamburg, Germany) were placed above (~150 cm) and in front (~40 cm) of the arena. Acoustic stimuli were presented through an ultrasonic loudspeaker (ScanSpeak, Avisoft Bioacoustics, Berlin, Germany), using an external sound card with a sampling rate of 192 kHz (Fire Wire Audio Capture FA-101, Edirol, London, UK) and a portable ultrasonic power amplifier having a frequency range of 1–125 kHz (Avisoft Bioacoustics). The loudspeaker, which has a frequency range of 1–120 kHz with a relatively flat frequency response (±12 dB) between 15 and 80 kHz, was placed 20 cm away from the observation arena. An additional, but inactive ultrasonic loudspeaker was arranged symmetrically at the opposite side as a visual control.

2.9. Acoustic stimuli

Rats were exposed to playback presentations of 50-kHz USV, time- and amplitude-matched white noise (NOISE), and background noise (BACKGROUND), but also SILENCE. NOISE and BACKGROUND were presented to control for unspecific effects not linked to the socio-affective communicative function of 50-kHz USV. All stimuli were presented with a sampling rate of 192 kHz in 16 bit format at ~69 dB (measured from a distance of 40 cm), with the exception of background noise, which was presented at ~50 dB, i.e. corresponding to the intensity of background noise present in the other acoustic stimuli.

A - 50-kHz USV: The 50-kHz USV had been recorded from an adult male Wistar rat during exploration of a cage containing scents from a cage mate after being separated from it (for setting and recording, see Willuhn et al., 2014). The acoustic stimulus material was composed of a sequence lasting 3.5s, which was presented in a loop. Each sequence contains 13 50-kHz calls (total calling time: 0.90s), with 10 of them being frequency-modulated and 3 flat.

B - NOISE: The artificial time- and amplitude-matched white noise was generated with SASLab Pro (Version 4.2, Avisoft Bioacoustics). Specifically, each given 50-kHz USV in the original natural 50-kHz USV stimulus material was replaced by white noise with durations and amplitude modulations identical to those of the original 50-kHz USV. Thus, the stimulus series had the same temporal patterning and was identical to the original natural 50-kHz USV series with respect to all call features, apart from the fact that sound energy was not confined to a certain frequency as in case of the natural 50-kHz USV.

C - BACKGROUND: Since the 50-kHz USV stimulus contained background noise, i.e. noises, which occur when a rat is exploring an arena with bedding, background noise without 50-kHz USV was presented.

D - SILENCE: Rats were not exposed to any acoustic stimulus during this phase of testing, i.e. they were submitted to the catalepsy test only.

2.10. Behavioral analysis

Additionally to the catalepsy time, and in order to investigate whether all acoustic stimuli were perceived by the rats, we assessed two behaviors while the rat remained with its forepaws on the bar during playback: (i) pinna reflex, a rapid and intermittent pinna movement, used to study the integrity of the lower auditory pathway (Horta-Júnior et al., 2008); and (ii) immediate head movement toward the sound source. The number of rats displaying these behaviors was counted. In cases where the animals stepped down from the bar and started to explore the arena, we quantified this exploratory behavior by dividing the arena into a proximal and a distal zone, i.e. one close to and the other opposite from the sound source, respectively, and each zone (24% of the arena) was further divided into 6 quadrants. Exploratory behavior was then assessed by counting quadrant crossings, defined as when the rat crossed a quadrant with its four paws.

2.11. Perfusion and histology

Upon completion of the experiments, the animals were deeply anesthetized with sodium pentobarbital (300 mg/kg) and perfused intracardially with physiological saline solution followed by formalin solution (10%). The midbrains were quickly removed and immersed in fresh fixative solution. After fixation, the brains were frozen and 50 µm serial brain sections were cut using a microtome. The sections were stained with cresyl violet in order to locate the positions of the cannula tips, according to the atlas of Paxinos and Watson (2007).

3. Statistical analysis

Effects of intracollicular NMDA - Experiment 1: ANOVAs for repeated measurements were performed, the first two ones with the within-subject-factor dose in NMDA and saline treated animals, followed by an unpaired-sample *t*-test (2-tailed), and the other

ones with within-subject-factor acoustic stimulus in NMDA and saline treated animals, followed by Student-Newman-Keuls post-hoc test when appropriate. In addition, one separate unpaired-sample *t*-test (2-tailed) was used to compare the catalepsy time between the NMDA and saline group during SILENCE, 50-kHz USV, NOISE and BACKGROUND playback presentation.

Effects of intracollicular diazepam - Experiment 2: ANOVAs for repeated measurements were performed: A first one with the within-subject-factor dose in diazepam and vehicle treated animals, followed by Student-Newman-Keuls post-hoc test when appropriate, and the second one with the within-subject-factor acoustic stimulus in diazepam and vehicle treated animals, followed by Student-Newman-Keuls post-hoc when appropriate. In addition, an unpaired-sample *t*-test (2-tailed) was used to compare the catalepsy time between the diazepam and vehicle group during SILENCE, 50-kHz USV, NOISE and BACKGROUND playback presentation. Statistical analyses were performed using IBM SPSS software Statistics 22. A p-value of <0.05 was considered statistically significant.

4. Results

4.1. Experiment I: intracollicular NMDA prevents paradoxical kinesia induced by 50-kHz USV

Replicating our own previous findings (Melo et al., 2010), NMDA (30 nmol) microinjected directly into the IC potentiated haloperidol–induced catalepsy as compared to saline under conditions without playback presentation (main effect drug treatment: $F_{1,6} = 6.30$, $P < 0.035$; time x drug treatment: $F_{1,6} = 6.30$, $P < 0.05$; Fig. 1A and B). This effect of NMDA was also seen in the second part of the study with playback presentation (main effect drug treatment: $F_{1,12} = 32.78$, $P < 0.001$). Furthermore, the effects of acoustic stimulus presentation on haloperidol-induced catalepsy were affected by NMDA treatment (main effect stimulus: $F_{3,36} = 2.49$, $P = 0.076$; stimulus x drug treatment interaction: $F_{3,36} = 3.58$, $P = 0.023$). In rats, that had received saline microinjections into the IC, there was an acoustic stimulus effect on catalepsy time (main effect stimulus: $F_{3,20} = 3.56$, $P = 0.032$). Specifically, playback of 50-kHz USV reduced haloperidol-induced catalepsy when compared to all other stimuli (NOISE, $t_5 = 2.70$, $P < 0.043$; BACKGROUND,

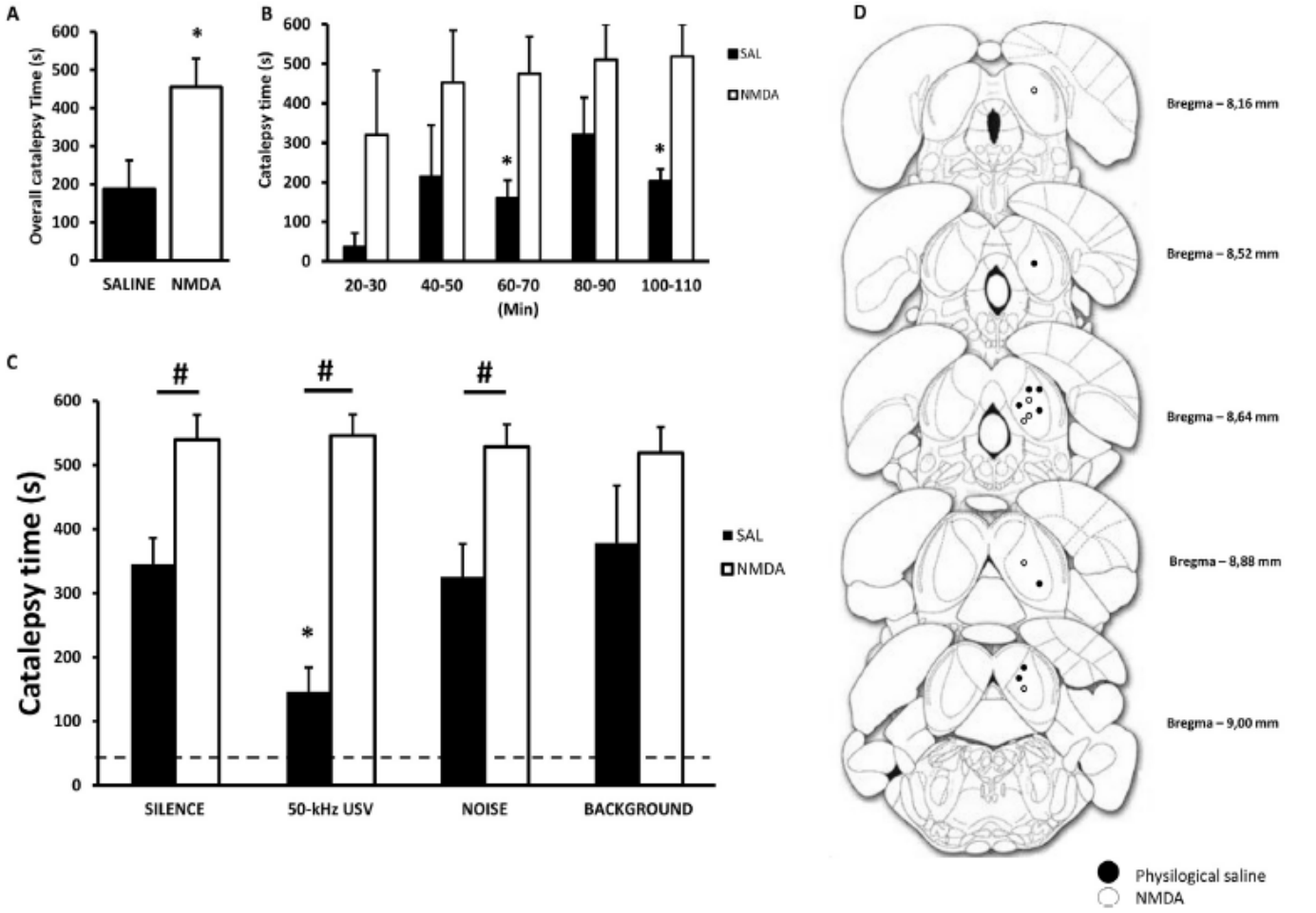

Fig. 1. (a) Effects of intracollicular NMDA on catalepsy time. Overall effects of intracollicular NMDA (30nmol/0.5 μl) or saline on rats that had received haloperidol systemically (0.5 mg/kg, IP). *P < 0,05 as compared with saline group. **(b)** Bars represent step-down latencies during 20–30, 40–50, 60–70, 80–90, 100–110 min after intracollicular administration of NMDA (30nmol/0.5 μl) or saline on rats that had received haloperidol systemically (0.5 mg/kg, IP). Data are expressed as means + SEM; (Sal + Halo group, N = 4; NMDA + Halo group, N = 4). *P < 0,01 as compared with NMDA group. **(c) Effects of intracollicular NMDA on catalepsy time during playback presentation.** Bars represent step-down latencies during SILENCE, 50-kHz USV, NOISE and BACKGROUND playback presentation on rats that had received intracollicular microinjection of NMDA (30nmol/0.5 μl) or saline and haloperidol systemically (0.5 mg/kg, IP). Data are expressed as means + SEM; (NMDA + Halo group, N = 8; SAL + Halo, N = 6). *P < 0.05 as compared with SILENCE, NOISE and BACKGROUND. #P < 0.05 indicates difference between Saline (SAL) and NMDA. Dashed line represents the criterion of 30s to start playback presentation. **(d) Microinjection placements.** Locations of microinjection placements in the inferior colliculus displayed on cross-sections from the atlas of Paxinos and Watson[26]. White and black dots indicate the placement of NMDA or Saline microinjections sites, respectively.

$t_5 = 2.60$, $P < 0.048$, or SILENCE $t_5 = 4.00$, $P < 0.010$), while no such effect was observed in case of NOISE or BACKGROUND stimuli or during SILENCE ($P > 0.050$; Fig. 1C). A similar responsiveness to playback 50-kHz USV during haloperidol-induced catalepsy had been demonstrated in our prior study (Tonelli et al., 2018). This catalepsy-reducing effect of 50-kHz USV playback was prevented by intracollicular microinjection of NMDA, since the catalepsy time did not differ between the different kinds of playback presentation for the NMDA group (main effect stimulus: $F_{3,20} = 0.106$, $P = 0.956$).

The analysis of pinna reflex and head movements (Table 1) showed that the pinna reflex was observed in all saline- and NMDA-injected animals exposed to playback of 50-kHz USV, NOISE, and BACKGROUND, compared to only occasional cases in the SILENCE condition. Regarding head movements, these were observed in all animals exposed to playback of 50-kHz USV, and most animals exposed to playback of NOISE, whereas no head movements occurred in case of BACKGROUND or SILENCE. The final histological analysis (Fig. 1D) showed that the microinjection sites were placed within the central nucleus of the IC.

4.2. Experiment II: intracollicular diazepam does not prevent paradoxical kinesia induced by 50-kHz USV

Under conditions without playback presentation, intracollicular administration of diazepam potentiated catalepsy induced by haloperidol (main effect drug treatment: $F_{4,108} = 18.54$, $P < 0.001$; time x drug treatment: $F_{2,27} = 7.04$, $P < 0.005$; Fig. 2A and B). However, during playback presentation, there was an effect of acoustic stimulus presentation on haloperidol–induced catalepsy as well as an effect of intracollicular diazepam microinjection (main effect stimulus: $F_{3,78} = 16.12$, $P < 0.001$; main effect drug treatment: $F_{1,26} = 7.25$, $P < 0.005$; stimulus x drug treatment interaction: $F_{6,78} = 1.07$, $P < 0.001$). In the animals, that had received vehicle microinjection into the IC, there was an acoustic stimulus effect on catalepsy time (main effect stimulus: $F_{3,33} = 5.92$, $P = 0.002$). Specifically, playback of 50-kHz USV reduced haloperidol-induced catalepsy when compared to all other stimuli (NOISE, $t_{11} = 3.53$, $P = 0.005$; BACKGROUND, $t_{11} = 3.60$, $P = 0.004$; SILENCE, $t_{11} = 3.48$, $P = 0.005$), while no such effect was observed in case of NOISE or BACKGROUND stimuli or during SILENCE ($P > 0.05$; Fig. 2C). Once more, these results support our findings seen in our prior study (Tonelli et al., 2018). Surprisingly, even though diazepam microinjected into the IC potentiated catalepsy induced by haloperidol, in rats treated with the higher dose of diazepam, there was a prominent effect of acoustic stimulus presentation (main effect stimulus: $F_{3,24} = 11.49$ $P < 0.001$): Catalepsy time was reduced in response to 50-kHz USV playback as compared to the other stimuli (NOISE $t_8 = 2.91$, $P = 0.019$; BACKGROUND $t_8 = 4.47$, $P = 0.002$) or SILENCE ($t_8 = 4.30$, $P = 0.003$; Fig. 2C). Moreover, in rats treated with the lower dose of diazepam, the playback of 50-kHz USV induced paradoxical kinesia when compared to SILENCE ($t_7 = 2.37$, $P = 0.049$).

The analysis of pinna and head movements (Table 2) showed that pinna movements were observed in all vehicle- and diazepam-injected animals exposed to playback of 50-kHz USV, NOISE, and BACKGROUND, however it was not seen during the SILENCE condition. Head movements were observed in most of the animals with playback of 50-kHz USV and NOISE, whereas fewer head movements occurred in case of BACKGROUND and none during SILENCE. All the microinjection sites were placed within the central nucleus of the IC (Fig. 2D).

Table 1
Percentage of rats displaying pinna reflex and immediate head movement (HM) towards the sound source.

	SIL	50-kHz	WN	BGN
Pinna Reflex (SAL)	33,33%	100%	100%	100%
Pinna Reflex (NMDA)	12,50%	100%	100%	100%
Immeadiate HM (SAL)	0%	100%	100%	0%
Immeadiate HM (NMDA)	0%	100%	71%	0%

5. Discussion

In the present study, we investigated the neural mechanisms underlying paradoxical kinesia, an intriguing phenomenon in which akinetic patients - when properly stimulated - accomplish tasks that they are otherwise unable to perform. In order to reproduce such a phenomenon in rats, first akinesia must be induced. Catalepsy induced by systemic administration of haloperidol models the bradykinesia/akinesia and lack of spontaneous motor activity that is common in some PD patients. Haloperidol is a neuroleptic drug, which mainly acts by blocking DA D2 receptors in the striatum, where they can be generally found on GABAergic projection neurons and cholinergic interneurons (Johnson et al., 2014; Kharkwal et al., 2016).

The results of the present study show that intracollicular administration of the glutamate receptor agonist NMDA potentiated haloperidol-induced catalepsy in rats, in line with our previous results (Melo et al., 2010; Medeiros et al., 2014). These findings strengthen the assumption that glutamate-mediated mechanisms in the neural circuits at the IC level can influence a motor impairment induced by impaired nigrostriatal DAergic neurotransmission. Along that line, and to the best of our knowledge, this is the first time showing that unilateral intracollicular diazepam can potentiate catalepsy induced by systemic haloperidol. This result corroborates our previous study, demonstrating that haloperidol-induced catalepsy can be potentiated by intracollicular administration of the GABAergic agonist midazolam (Tostes et al., 2013). Although opposite effect of GABAergic and glutamatergic agonists would be expected, these apparently contradictory results may be explained by activation of different projections. For instance, the IC sends direct glutamatergic projections to pontine nuclei (pontocerebellar auditory pathway; Saint Marie, 1996) and GABAergic and glutamatergic projections to the medial geniculate body (MGB). Therefore, stimulation of these projections may underlie temporal patterns of inhibition and excitation. Moreover, electrophysiological studies suggested that IC inhibition may precede excitation under some conditions *in-vivo* (Bartlett and Smith, 1999).

Indeed, previous studies from our group have shown that intracollicular microinjection of the GABAergic antagonist bicuculline can produce a biphasic effect, from attenuation to potentiation of catalepsy induced by systemic haloperidol (Tostes et al., 2013). Systemic or intrastriatal haloperidol-induced catalepsy can be significantly reduced by prior microinjection of the NMDA glutamate receptor antagonist MK-801 into the IC (Melo et al., 2010; Medeiros et al., 2014). Together these results point to the IC as an important sensorimotor interface influencing haloperidol-induced catalepsy and suggest that GABAergic and glutamatergic neurotransmission in the IC are involved. Furthermore, we had recently shown that high-frequency deep brain stimulation of the IC reduced haloperidol-induced catalepsy, representing an animal model of paradoxical kinesia induced by aversive stimulation, since these stimulation parameters led to flight responses (Melo-Thomas and Thomas, 2015).

Regarding our present playback findings, it is important to note that auditory stimulation can induce paradoxical kinesia in humans, and recently, we demonstrated that playback presentation of natural 50-kHz USV reduced haloperidol-induced catalepsy in rats, representing an animal model to study paradoxical kinesia

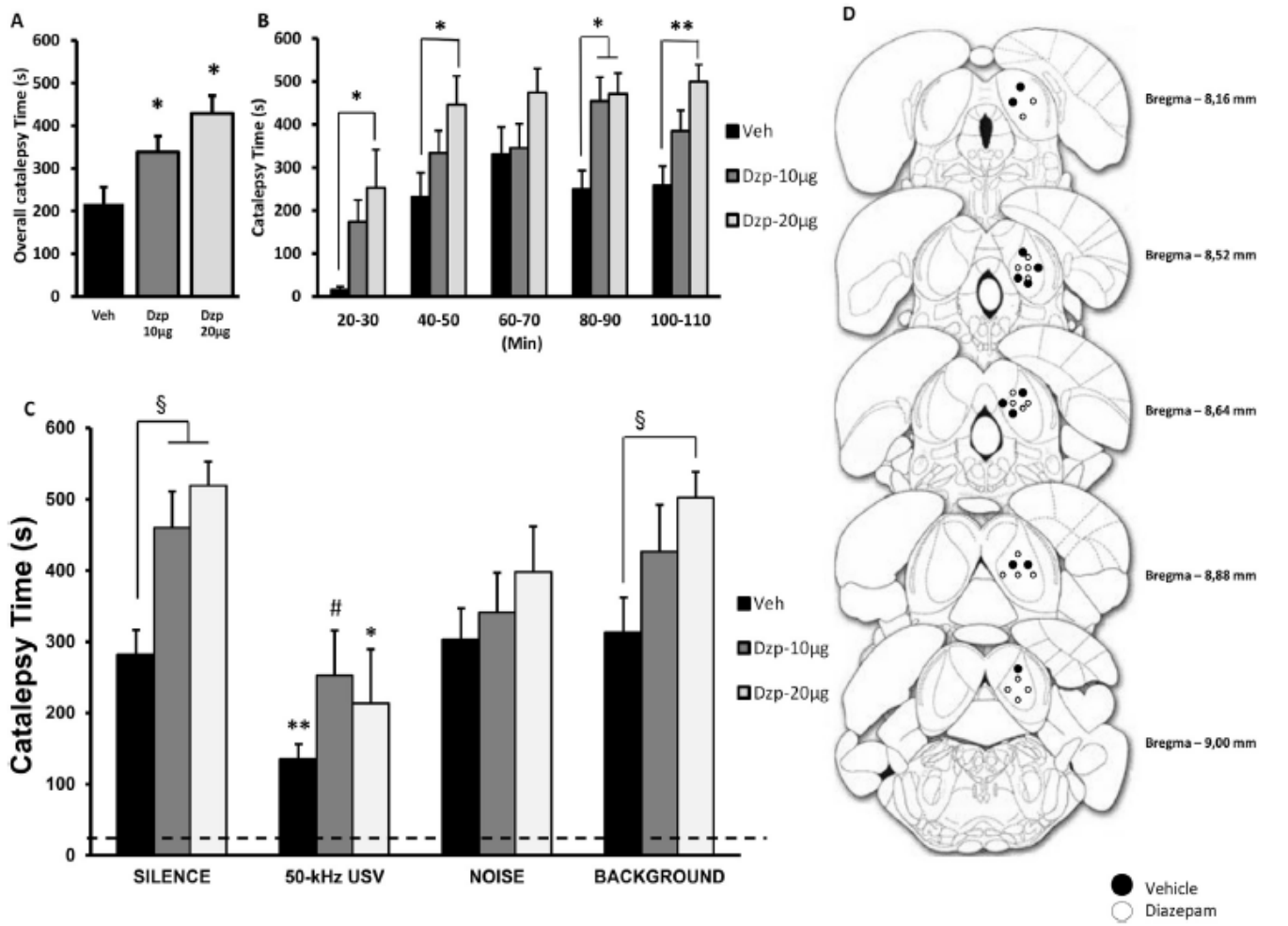

Fig. 2. (a) Effects of intracollicular diazepam on catalepsy time. Overall effects of intracollicular diazepam (10µg/0.5 µl and 20µg/0.5 µl) or vehicle on rats that had received haloperidol systemically (0.5 mg/kg, IP). *P < 0,05 as compared with vehicle group. **(b)** Bars represent step-down latencies during 20–30, 40–50, 60–70, 80–90, 100–110 min after intracollicular administration of diazepam (10µg/0.5 µl and 20µg/0.5 µl) or vehicle on rats that had received haloperidol systemically (0.5 mg/kg, IP). Data are expressed as means + SEM; (Veh + Halo group, N = 10; 10 mg + Halo group, N = 11; 20 mg + Halo, N = 9). *P < 0,01 and **P < 0,001 as compared with vehicle. **(c) Effects of intracollicular diazepam on catalepsy time during playback presentation.** Bars represent step-down latencies during SILENCE, 50-kHz USV, NOISE and BACKGROUND playback presentation on rats that had received intracollicular microinjection of diazepam (10µg/0.5 µl and 20µg/0.5 µl) or vehicle and haloperidol systemically (0.5 mg/kg, IP). Data are expressed as means + SEM; (Veh + Halo group, N = 12; 10 mg + Halo group, N = 8; 20 mg + Halo, N = 9). *P < 0,05 and **P < 0,01 as compared with SILENCE, NOISE and BACKGROUND. #P < 0,05 as compared with SILENCE. §P < 0,01 as compared with vehicle. Dashed line represents the criterion of 30s to start playback presentation. **(d) Microinjection placements.** Locations of microinjection placements in the inferior colliculus displayed on cross-sections from the atlas of Paxinos and Watson[27]. White and black dots indicate the placement of Diazepam or Vehicle microinjections sites, respectively.

Table 2
Percentage of rats displaying pinna reflex and immediate head movement (HM) towards the sound source.

	SIL	50-kHz	WN	BGN
Pinna Reflex (Vehicle)	0%	100%	100%	100%
Immediate HM (Vehicle)	0%	91.6	75%	58.3%
Pinna Reflex (Dzp-10µg/0.5 µl)	0%	100%	100%	100%
Immediate HM (Dzp-10µg/0.5 µl)	0%	100%	100%	62.5%
Pinna Reflex (Dzp-10µg/0.5 µl)	0%	100%	100%	100%
Immediate HM (Dzp-10µg/0.5 µl)	0%	100%	77.7%	22.2%

induced by appetitive acoustic stimulation (Tonelli et al., 2018). In the present study, we took a first attempt to disclose the neural mechanisms involved in such paradoxical kinesia induced by species-specific ultrasounds. We obtained support for an involvement of the IC, since the glutamatergic agonist NMDA enhanced not only haloperidol-induced catalepsy, in line with our previous results (Melo et al., 2010; Medeiros et al., 2014), but prevented the effectiveness of 50-kHz USV to induce paradoxical kinesia (Tonelli et al., 2018). As shown by the measures of pinna and head movements, this effect was obtained without a blockade of basic auditory processing, which are also mediated in structures outside the IC, like in the lower brainstem (Horta-Júnior et al., 2008; Henkel and Edwards, 1978). Indeed, the IC has projections to the deep and intermediate layers of the superior colliculus, which is responsible to control head, eye and pinna movements for orientation toward sounds and objects in space (Casseday and Covey, 1996). Despite this, the present IC microinjections did not affect these responses and the rats remained the entire time on the bar due to the effect of intracollicular microinjection of NMDA.

In respect of the effects of intracollicular NMDA on 50-kHz USV playback, it is important to highlight that the IC serves not only as an important auditory relay structure including tonotopic representations for ultrasonic frequencies (Malmierca and Merchán, 2004), but also sends afferents to pontine and medullary motor structures, and to the superior colliculus, and through that to the substantia nigra, thereby indirectly assessing the basal ganglia (Castellan-Baldan et al., 2006). Indeed, the IC is one of several brainstem sensorimotor structures, which are not only indirectly connected to the basal ganglia via sensorimotor loops, but also

structures where basal ganglia outputs converge into a final common motor path to generate behavioral output (Redgrave et al., 2010; Olazábal and Moore, 1989; Moriizumi and Hattori, 1991). Therefore, one can assume that external auditory stimulation may induce paradoxical kinesia by activation of motor circuits via the IC even when striatal DA transmission is impaired during neuroleptic-induced catalepsy (Melo et al., 2010; Melo-Thomas and Thomas, 2015; Medeiros et al., 2014), and that this phenomenon can be prevented by NMDA administration into the IC.

Although intracollicular diazepam potentiated the catalepsy induced by haloperidol, 50-kHz USV playback was still able to induce paradoxical kinesia, i.e. rats were released from catalepsy. Importantly, this effect was seen in rats treated with both doses of diazepam, but the effect was more prominent in case of the higher dose. The fact that both NMDA and diazepam potentiate haloperidol-induced catalepsy but that only NMDA counteracts paradoxical kinesia induced by 50-kHz USV playback presentation may indicate that this phenomenon requires a low level of glutamate in the IC. Indeed, the microinjection of the glutamatergic antagonist MK-801 into the IC induces paradoxical kinesia when no USV is involved (Melo et al., 2010; Medeiros et al., 2014). Still, 50-kHz USV-induced paradoxical kinesia was not affected by increasing GABAergic neurotransmission in the IC. Together, the present results suggest that the neurobiological mechanisms underlying paradoxical kinesia through the IC may be glutamatergic rather than GABAergic. Importantly, the basic auditory processing was not affected by the intracollicular microinjection of diazepam since the measures of pinna reflex and immediate head movement showed that the rats were perceiving all the acoustic signals during the catalepsy test.

Interestingly, we had previously found that auditory 50-kHz USV stimuli, as used here, lead to phasic DA release in the nucleus accumbens (Willuhn et al., 2014), which probably underlies its approach-eliciting effects in intact animals. Possibly, such an effect also plays a role in case of akinetic animals which have received haloperidol (Tonelli et al., 2018), since paradoxical kinesia might be mediated either by DA reserves within the basal ganglia, or by routes by-passing them (Glickstein and Stein, 1991). It remains to be shown, however, whether such effects are also possible in case of rather complete blockade of D2 receptors as used here, especially since it is thought that paradoxical kinesia is not mediated by actions on striatal DA (Keefe et al., 1989).

How might the IC influence haloperidol-induced catalepsy? When no emotional or motivational state is involved, i.e. the cataleptic state without additional 50-kHz USV playback, both glutamatergic and GABAergic agonists microinjected into the IC potentiated haloperidol-induced catalepsy probably by influencing descending auditory-motor pathways. Indeed, the pedunculopontine nucleus (PPN), and the cuneiform nucleus (CnF), which form the neuroanatomical basis of the mesencephalic locomotor region (MLR; Takakusaki, 2017), receive direct projections from the IC. Recently it has been suggested that glutamatergic PPN activity may facilitate slow, explorative locomotor behavior whereas those in the CnF promote escape locomotion (Caggiano et al., 2018). In addition, the MLR sends neurons to the reticular formation (RF), that are thought to mediate locomotion (Jordan et al., 2008) and from there to the spinal cord that executes locomotion (Kiehn, 2016; see Fig. 3).

However, when an emotional and motivational state is involved, i.e. the cataleptic state with additional 50-kHz USV playback, an auditory-amygdalar feedback may be recruited. In fact, the medial geniculate body (MGB) that receives major input from the IC, sends outputs to the amygdala (LeDoux et al., 1985). In addition, a direct projection from the basal nucleus of the amygdala was found with terminals distributed widely throughout the IC, including most of the central nucleus (ICC), i.e. the major recipient of ascending auditory brainstem input (Marsh et al., 2002). Additional projections from the amygdala and the auditory cortex to the IC have been reported (Marsh et al., 2002; see Fig. 3). The function of this projection is not known, but the presence of such an auditory–amygdalar feedback circuit involving the IC may modify the processing of sound early in the ascending auditory pathway on the basis of an animal's emotional or motivational state. Possibly, this projection could explain our results since glutamatergic, but not GABAergic, intracollicular mechanisms may be involved in this auditory-amygdalar feedback affecting the motor response (catalepsy) to a highly emotional and motivational auditory stimulus, i.e. 50-kHz USV playback. Ultimately, this auditory-amygdalar feedback may modulate the descending projection from the IC to the MLR to ensure an appropriate motor response.

The present findings may have relevance for diseases and disorders, where catalepsy and akinesia are prominent, and where paradoxical kinesia is observed. Here, PD seems to be a likely candidate, since PD patients are thought to have intact motor

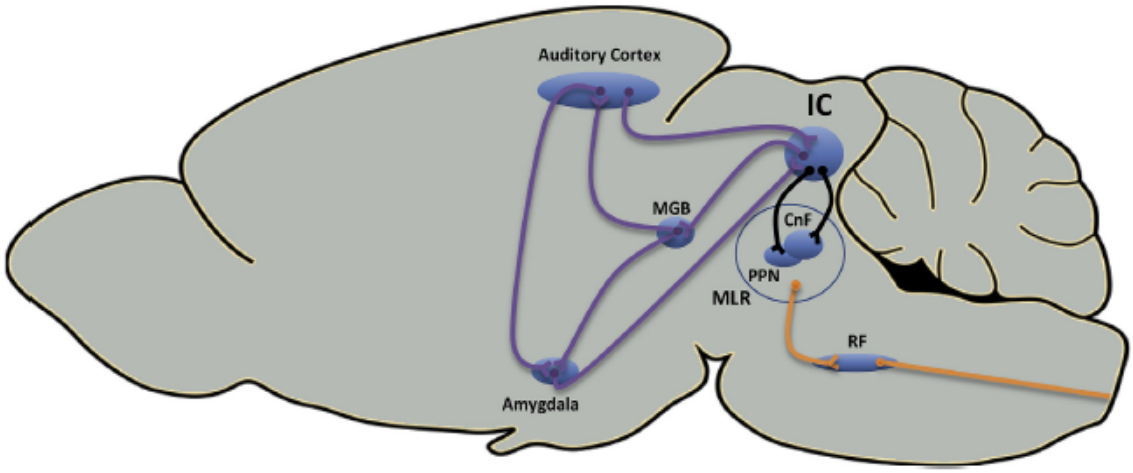

Fig. 3. Pathways by which the inferior colliculus (IC) may influence motor behavior and therefore induce paradoxical kinesia. Black lines indicate projections from the inferior colliculus (IC) to the cuneiform nucleus (CnF) and to the pedunculopontine nucleus (PPN), which are both considered as constituent of the mesencephalic locomotor region (MLR; Takakusaki, 2017). Orange lines indicate neurons from MLR to the reticular formation (RF), that are thought to mediate locomotion (Jordan et al., 2008), and from there to the spinal cord that execute locomotion (Kiehn, 2016). Purple lines indicate projections from the IC to the medial geniculate body (MGB) followed by its projections directly to the amygdala (LeDoux et al., 1985) and auditory cortex. In turn, the IC receives projections from both amygdala and auditory cortex representing the auditory-amygdalar feedback (Marsh et al., 2002). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

programs but have difficulty accessing them without an external trigger (Jankovic, 2008; Glickstein and Stein, 1991; Clark et al., 2009). Regarding such triggers, auditory and visual stimulation were found to be effective, which possibly act due to activation of alternative pathways than those of the basal ganglia, for example, via cerebellar circuits, or by activation of DAergic reserves within the basal ganglia (Clark et al., 2009). With respect to alternative pathways, our present and previous work suggests that the IC may provide a critical site, which can activate motor circuits. Since we used appetitive auditory stimulation, our model of paradoxical kinesia may especially be relevant to clarify the neural mechanisms underlying the benefits of auditory stimulation in PD patients, (Rubinsten et al., 2002; Arias and Cudeiro, 2008).

Relevant conflicts of interests/financial disclosures

No conflicts of interest/financial disclosures.

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6 APPENDIX

6.1 Abbreviations

µg	microgram
µl	microliter
AP7	2-amino-7-phosphonoheptanoic acid
BZD	Benzodiazepine
CNF	Cuneiform nucleus
DA	Dopamine
dB	decibel
FOG	Freezing of gait
GABA	Gamma-Aminobutyric Acid (γ-Aminobutyric acid)
Hz	Hertz
IC	Inferior Colliculus
ICC	Inferior Colliculus Central Nucleus
Kg	Kilogram
kHz	Kilohertz
L-Dopa	L-3,4-dihydroxyphenylalanine (levodopa)
L-NOARG	N(G)-nitro-L-arginine
mg	milligram
Mk-801	(5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine
MLR	Mesencephalic Locomotor Region
NMDA	1-methyl-4- phenyl-1, 2, 3, 6-tetrahydropyridine
Nmol	Nanomole
NOS	Nitric Oxide Synthase
PD	Parkinson`s disease
PPN	Pedunculopontine Nucleus
SNpr	Substantia nigra pars compacta
USV	Ultrasonic Vocalizations

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9 CURRICULUM VITAE

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PUBLICATIONS

- **Tonelli LC**, Wöhr M, Schwarting R, Melo-Thomas L. Paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats depends on glutamatergic mechanisms in the inferior colliculus. **Neuropharmacology.** 2018 Mar 14;135:172-179.
- **Tonelli LC**, Wöhr M, Schwarting R, Melo-Thomas L. Awakenings in rats by ultrasounds: A new animal model for paradoxical kinesia. **Behav Brain Res.** 2018 Jan 337;204-209.
- Medeiros P, Viana MB, Barbosa-Silva RC, **Tonelli LC**, Melo-Thomas L. Glutamatergic neurotransmission in the inferior colliculus influences intrastriatal haloperidol-induced catalepsy. **Behav Brain Res.** 2014 Jul 15;268:8-13.

PUBLICATIONS IN SUPPLEMENTS

- **Tonelli LC**, Wöhr M, Schwarting R, Melo-Thomas L. "P.2.008 - Paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalisations in rats depends on glutamatergic

mechanisms in the inferior colliculus". European Neuropsychopharmacology, Volume 28, Supplement 1, March 2018, Pages S26-27.

- Tavares, P., Pinto-Pereira, S., Moretto, D., Lopes, A., Paiva, A., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, "The influence of aerobic exercise on the number and differentiation of blood rat endothelium progenitor cells (EPC s)". J Tissue Eng Regen Med, Volume 6, Supplement 2, October, 2012, Pages 8-39.

HONORS AND AWARDS

- Scholarship for **full Doctorate** – Coordination for the Improvement of Higher Education Personnel (CAPES); 2014 - 2018, Brazil – Germany.
- **IBNS Travel Awards 2018** for "Glutamatergic mechanisms in the inferior colliculus play a key role in paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats". June – 2018, Florida, United States of America.
- Award junior scientist in Europe by the **ECNP Workshop** on Neuropsychopharmacology. March – 2018, Nice, France.
- Award best poster presentation at the **"Donders Discussion 2017**" "Awakenings in cataleptic rats by ultrasounds: a new animal model for paradoxical kinesia and its possible". October – 2017, Nijmegen, Netherlands.
- **ECNP Travel Award 2017** for "Awakenings in cataleptic rats by ultrasounds: a new animal model for paradoxical kinesia and its possible. September – 2017, Paris, France.
- Award by the **German neuroscience society for young investigator** at the 12th Göttingen Meeting of the German Neuroscience Society. Oral talk presented on *"*Parkinsonian rats respond to ultrasonic vocalizations: a new animal model of paradoxical kinesia". March – 2016, Göttingen, Germany.
- Scholarship for **Erasmus researcher** – Santander Bank; 2012 – 2013, Brazil – Portugal.
- Scholarship for **beginner researcher** – São Paulo Research Foundation (FAPESP); 2011 – 2012, Brazil.

ORAL PRESENTATIONS

- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "Glutamatergic mechanisms in the inferior colliculus play a key role in paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats". International behavioral Neuroscience Society – IBNS. June – 2018, Florida, USA.
- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "Parkinsonian rats respond to ultrasonic vocalizations: a new animal model of paradoxical kinesia". 12th Göttingen Meeting of the German Neuroscience Society. March – 2016, Göttingen, Germany.
- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "The influence of auditory stimulation on motor impairments in an animal model of parkinsonism". Seminar for PhD students from Brazil. September – 2015, Bonn, Germany.

POSTER PUBLICATIONS

- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "Glutamatergic mechanisms in the inferior colliculus play a key role in paradoxical kinesia induced by appetitive 50-kHz ultrasonic vocalizations in rats". International behavioral Neuroscience Society – IBNS. June – 2018, Florida, USA.
- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, "50-kHz ultrasonic vocalizations can induce paradoxical kinesia in cataleptic rats: A new animal model and its possible mechanisms". Society for Neuroscience – SfN. November – 2017, Washington. USA.
- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "Awakenings in cataleptic rats by ultrasounds: a new animal model for paradoxical kinesia and its possible mechanism". Donders Discussion. October – 2017, Nijmegen, Netherlands.
- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "Awakenings in cataleptic rats by ultrasounds: a new animal model for paradoxical kinesia and its possible mechanism". ECNP Travel 2017 September – 2017, Paris, France.

- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "Awakenings in cataleptic rats by ultrasounds: a new animal model for paradoxical kinesia and its possible mechanisms". International behavioral Neuroscience Society – IBNS. June – 2017, Hiroshima, Japan.
- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, "Ultrasonic vocalizations release catalepsy in rats: a new animal model of paradoxical kinesia". 13th International Conference on Alzheimer's & Parkinson's Diseases – ADPD, March – 2017, Vienna, Austria.
- **Tonelli, L.C.,** Wöhr, M., Schwarting, R.K., Melo-Thomas, L, "The influence of auditory stimulation on motor impairments in an animal model of parkinsonism". Seminar for PhD students from Brazil. September – 2015, Bonn, Germany.
- Moretto, D., Pinto-Pereira, S., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, Tavares, Paula, "Characterization Of skeletal Muscle Fiber Type Changes Induced By aerobic exercise In Rat soleus Muscle". American College of Sports Medicine. 2013, USA.
- Moretto, D., Pinto-Pereira, S., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, Tavares, Paula, "Effect of aerobic exercise in rat soleus muscle. Implications of NOs and VEGF". V Annual Meeting of IBILI. 2012, Coimbra, Portugal.
- Boieiro, A., Mogas, F., Moretto, D., **Tonelli, L.,** Tavares, P., André, A., Paulo, G, "Effect of aerobic exercise in rat gastrocnemius architecture". IV Annual Meeting of IBILI. 2012, Coimbra, Portugal.
- Tavares, P., Pinto-Pereira, S., Moretto, D., Lopes, A., Paiva, A., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, "Exercise induces mobilization and differentiation of circulating rat endothelial progenitor cells (epcs)". IV Annual Meeting of IBILI. 2012, Coimbra, Portugal.
- Tavares, P., Pinto-Pereira, S., Moretto, D., Lopes, A., Paiva, A., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, "Mobilization and differentiation of circulating rat endothelial progenitor cells (EPCs) after aerobic exercise training". II Congresso Luso-Brasileiro de Patologia Experimental. 2012, Coimbra, Portugal.
- Moretto, D., Pinto-Pereira, S., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, Tavares, Paula, "Effect of aerobic exercise in rat soleus muscle. Implications of NOs and VEGF". II Congresso Luso-Brasileiro de Patologia Experimental. 2012, Coimbra, Portugal.

- Tavares, P., Pinto-Pereira, S., Moretto, D., Lopes, A., Paiva, A., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, The influence of aerobic exercise on the number and differentiation of blood rat endothelium progenitor cells (EPC s). TERM STEM. October – 2012, Guimães, Portugal.
- Tavares, P., Pinto-Pereira, S., Moretto, D., Lopes, A., Paiva, A., **Tonelli, L**., André, A., Fontes Ribeiro, C. A, The influence of aerobic exercise on the number and differentiation of blood rat endothelium progenitor cells (EPC s). Effect of simultaneous treatment with ibuprofen. TERM STEM. October – 2012, Guimães, Portugal.

EDUCATION

2014 - Present	Doctorate student at Philipps - University of Marburg, Experimental and Physiological Psychology, Marburg, Germany. **Title of the thesis:** "A new animal model of paradoxical kinesia induced by 50-kHz ultrasonic vocalizations playback in rats: implications of the inferior colliculus"
2012	Erasmus Studies at Faculty of Sport Sciences and Physical Education, Coimbra, Portugal.
2009 – 2013	Graduate Physical Education - Health Sciences Mode; Federal University of São Paulo, Brazil.
2004 – 2006	High School Diploma at Escola Dr. Ernane Vilela Lima, Nepomuceno – MG, Brazil.

PEER-REVIEWER EXPERIENCE

- Brain Research;
- Neurobiology of Disease.

RESEARCH EXPERIENCE

2014 - Present	The involvement of the inferior colliculus in the catalepsy induced by haloperidol in rats. Behavioral and electrophysiological study. **Department of Psychology, Philipps - University of Marburg.**
2012	Analysis of mechanisms underlying the modulation of fiber muscle type (II) to the type (I) induced by exercise. **Faculty of Sport Sciences and Physical Education and Department of Pharmacology and Experimental Therapeutics (IBILI - Faculty of Medicine, University of Coimbra) Coimbra, Portugal.**
2011 – 2013	The role of the glutamatergic receptors in the inferior colliculus in the catalepsy induced by haloperidol. **Federal University of São Paulo, Brazil**.
2009 – 2010	Effects of sport games on balance and physical skills of elderly obese women. **Federal University of São Paulo, Brazil.**

EXPERIENCE TECHNIQUES

- Stereotaxic surgery in rodents;
- Transcardial perfusion in rodents;
- Deep Brain Stimulation;
- Histology (Microtome; Nissl Staining);
- Blood withdrawal;
- Suturing;

- Western blot.

MEMBERSHIPS

- International Behavioral Neuroscience Society;
- German Neuroscience Society;
- Marburg Research Academy (MARA).

PROFESSIONAL TRAINING

- Course on Laboratory Animal Science – FELASA category B (40 hours); *Provadis Partner für Bildung & Beratung*, (2014);
- Thomas RECORDING GmbH microdrive Technique (electrophysiology), Gießen, Germany (2012).

10 EIDESSTATTLICHE ERKLÄRUNG [DECLARATION OF ACADEMIC HONESTY]

Ich versichere, dass ich meine Dissertation

“A new animal model of paradoxical kinesia induced by 50-kHz ultrasonic vocalizations playback in rats: implications of the inferior colliculus”

selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderer als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Marburg, im Mai 2018

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Luan Castro Tonelli