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Research report

Neuronal activity in the bed nucleus of the stria terminalis in a rat model for obsessive–compulsive disorder

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HIGHLIGHTS

- We evaluated the neuronal activity in the bed nucleus of the stria terminalis in rats.
- We used an animal model for obsessive–compulsive disorder.
- We compared activity between schedule-induced polydipsia rats, resistant, and control rats.
- Firing rate and firing pattern parameters differ between these groups.
- Firing pattern parameters differ between hemispheres and position within the nucleus.

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ABSTRACT

In search of a new potential target for deep brain stimulation in patients with obsessive–compulsive disorder (OCD), we evaluated the single-cell activity of neurons in the bed nucleus of the stria terminalis (BST) in urethane-anesthetized rats in an animal model for OCD, the schedule-induced polydipsia (SIP) model, and compared this to the BST activity in control rats and to a third group of rats which were introduced in the model but did not develop the SIP, and thus were considered resistant. We compared the firing rate and firing pattern of BST neurons between these groups, between hemispheres and made a correlation of the firing rate and firing pattern to the position in the BST. The variability of BST neurons in SIP rats was lower and the randomness higher than BST neurons in control rats or resistant rats. The firing rate of BST neurons in SIP rats was significantly higher and the burst index lower than BST neurons in resistant rats but not in control rats. Also, neurons from the right hemisphere in the SIP group had a higher burst index than neurons from the left hemisphere. However, this is opposite in the resistant and control group. Third, we found a higher bursting index with increasing (more ventral) depth of recording. These findings suggest that schedule-induced polydipsia, which models compulsive behavior in humans, induces a change in firing behavior of BST neurons.

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1. Introduction

Obsessive–compulsive disorder (OCD), a psychiatric disorder with a prevalence of 0.8% in adults and 0.25% in 5–15 year old children [1,2], is characterized by anxiety, obsessions (persistent intrusive thoughts), and compulsions (e.g. checking, ordering, and counting), which can cause significant impairment of the patients. For a substantial portion of the patients, current pharmacological and behavioral therapies do not relieve symptoms sufficiently [3]. Neurosurgical techniques such as capsulotomies or deep brain stimulation (DBS) in the anterior limbs of the internal capsule and the ventral striatum may be a last resort investigational treatment [4,5]. Although these therapies may have a good outcome, little

Abbreviations: OCD, obsessive–compulsive disorder; SIP, schedule-induced polydipsia; CON, control; RES, resistant; BST, bed nucleus of the stria terminalis; DBS, deep brain stimulation.

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is still known about the underlying neurological mechanisms of the disorder and these therapies. New insights could be gained by investigating the neuronal activity in these brain regions.

Casual reports have described the efficacy of DBS in the bed nucleus of the stria terminalis (BST), a gray matter brain structure just below the anterior limbs of the internal capsule [6]. The efficacy of DBS in this brain structure seemed to be strongly related to the position of the electrode to the BST [7]. This nucleus has connections with the cortico-striato-thalamo-cortical circuitry [8] and neuroimaging studies demonstrated the importance of this circuitry in the pathophysiology of OCD [9].

Based on these observations, we set out to investigate the neuronal single-cell activity in the BST in the schedule-induced polydipsia (SIP) model, a validated animal model for obsessive–compulsive behavior [10,11]. In this model, hungry rats that receive a small food pellet every 60 s drink a physiologically excessive amount of water. This behavior resembles the compulsions in OCD patients because this behavior is repeated over and over again in an often useless and harmful way (face validity). The SIP creates a scenario, which fits the criteria for compulsive behavior, it induces stereotyped excessive performance and resistance to both extinction and consummation [12]. In addition, the SIP model has predictive validity since drugs that efficiently reduce OCD symptoms, like fluoxetine, fluvoxamine, and clomipramine, diminish water intake in the model, while other drugs, that have no effect on OCD symptoms, like haloperidol and diazepam, do not have an effect in the model [10]. Furthermore, previous work showed the efficacy of high-frequency electrical stimulation in the BST in this model to reduce the water intake [13].

The objectives of this study were to compare the neuronal firing rate and firing pattern (1) between SIP rats, rats that are resistant to the model, and normal control rats, (2) between the left and right hemisphere in each of the three groups (since, in some cases, unilateral deep brain stimulation is sufficient to relieve a patients' symptoms [14,15]) and (3) to correlate the neuronal firing rate and firing pattern with the recording position within the BST in each of the three groups.

2. Methods and materials

2.1. Subjects and housing

Forty-four young male Wistar rats were used (±200 g at arrival) and housed in pairs under controlled temperature and lightening conditions with food and water available ad libitum. All experiments were carried out in accordance with protocols approved by the local university animal ethics committee and in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

2.2. Schedule-induced polydipsia model and experimental groups

Rats were randomly allocated to one of two groups: the polydipsia group ($n = 33$) or the control group ($n = 11$). After a one-week acclimatization period, food was restricted until rats reached 85% of their original body weight. The rats were then subjected to 15 preoperative schedule-induced polydipsia tests on week days. Rats were placed in test chambers and a dispenser automatically delivered one 45-mg pellet (dustless precision pellets, #F0021-J, Bioserv, Frenchtown, NJ, USA) on a fixed-time 60 s feeding schedule for 30 min test sessions. To assess schedule-induced polydipsia, water intake (g) was measured by weighing the water bottles before and after the 30 min test sessions. Water bottles with a ball bearing in the tube prevented water loss by spontaneous dripping. Every day the order of the subject testing was random. On the day the rat consumed 8 ml water or more [13], the rat was considered polydiptic (SIP group). Control rats (CON group) were tested in the same environment but received all the 30 food pellets at once and were pair grouped with a rat from the SIP group. If a rat, subjected to the SIP model, did not meet the 8 ml criterion after 15 test days, the rat was considered resistant (RES group).

2.3. Electrophysiological recording

The rats were placed under general anesthesia (urethane 1.3 g/kg, i.p.). Local anesthesia (Xylocaine® s.c., 2% 20 mg/ml; AstraZeneca SA, Brussels, Belgium) was used before scalp incision after shaving and disinfecting with alcohol (1% in 70% isopropyl alcohol). The head was positioned in the stereotactic frame so that bregma and lambda were in the same horizontal plane.

A heating blanket controlled by an anal probe prevented hypothermia during the surgery. Rats received 0.4 ml amoxicillin (Duphamox® log active; Fort Dodge Animal Health Benelux BV, Wemmel, The Netherlands) and the eyes were protected from dehydration by polymyxine B (Terra-Cortril; N.V. Pfizer S.A., Brussels, Belgium). A longitudinal incision was made and the burr holes were drilled after visualization of bregma. The electrodes (Tungsten, microTargeting™ electrode mTDWBS26710262, 1 MΩ impedance, FHC Inc., Bowdoin, USA), attached to the stereotactic arms, were placed above the burr holes. After the dura was punctured with a needle, the electrodes were implanted with a microdrive (10 μm/s, D. Kopf Instruments®, Tujunga, USA) bilaterally in the bed nucleus of the stria terminalis (coordinates: 0.60 mm posterior to bregma, 1.3 mm lateral to the midline, and 5.2–7.2 mm ventral to the dura mater [16]). One insertion track per hemisphere was performed subsequently and choice of first hemisphere per rat was at random. When a possible neuron was encountered, signals were recorded for 5 min, amplified and band pass-filtered (500 Hz to 5 kHz) and digitized at 24 kHz with the Leadpoint (Medtronic) where it was stored for offline analysis.

2.4. Histology

After the experiment, the rats were euthanized with Nembutal (3 ml, i.p.; CEVA Santé Animale, Brussels, Belgium). Next, they were perfused intracardially with 10% sucrose in distilled water (5 min) and 4% formaldehyde in distilled water (15 min) followed by the resection of the brain. The brains were processed for paraffin coronal sectioning (5 μm) and brain slices were stained with hematoxylin–eosin and examined under a light microscope. If the location of the tip of the electrode was not in the BST, according to [16], the results of that recording were excluded from the analyses. Data coming from these neurons were used to assess whether the outcome was specific for BST neurons.

2.5. Data analysis

The analysis was performed blindly using the Wave_clus 2.0 package in Matlab (Mathworks Inc., Nattik, USA), based on superparamagnetic clustering [17], for extraction of individual neural clusters. Neurons were treated equally with no possible sub-group considerations. The output (spike clusters and timestamps) was combined with a custom made Matlab code to enable data processing [18]. Only stable neuronal activities (in the sense of observed firing during the entire recording period) with a signal-to-noise ratio above 1.7 (according to the definition in [19]) were used. To describe the firing properties of the neurons, we used the firing rate (spikes/s), the C_v coefficient, also called variability (=the standard deviation of the interspike intervals divided by the mean interspike interval), the burst index (=number of interspike intervals < 10 ms divided by the number of interspike intervals > 10 ms) [20–22], and the η coefficient or randomness (entropy measure, logically corresponding to the choice of different interspike intervals that appear in the spike train and the 'freedom' in their serial ordering, calculated similarly as suggested in [23]).

2.6. Statistical analysis

In order to correct for the dependency between multiple observations per rat, generalized estimating equations modeling with compound symmetry working correlation matrix was applied, instead of the commonly used ANOVA [24,25]. We used a logarithmic transformation on the firing rate and variability to have a more normal distribution, but this was not possible for the burst index and the randomness. The generalized estimating equations modeling was carried out using the SAS system release 9.2 (SAS Institute Inc., Cary, NC, USA). p-Values < 0.05 are considered significant. Data are depicted as mean ± standard error of the mean. However, caution should be taken in the interpretation of the standard error, as this measure of variability does not take into account the dependency between different observations of the same rat, and are shown here only for illustration.

3. Results

Seven out of 33 animals (21%) in the schedule-induced polydipsia model reached the 8 ml criterion after 15 test sessions and were considered polydiptic. After histological analysis and spike sorting, a total of 196 neurons recorded from the BST were used in the statistical analysis, with signals from 29 neurons from the SIP group ($n = 6$ rats), 114 neurons from the RES group ($n = 20$ rats), and 53 neurons from the CON group ($n = 6$ rats). Only signals in accordance with criteria described above were included in the analysis.

The number of recorded neurons per rat did not differ between the groups (Kruskal–Wallis, $p = 0.58$). Recording sites are depicted in Fig. 1. There was no difference in the position (subdural, mediolateral, or anterior-posterior) of the recording sites between the

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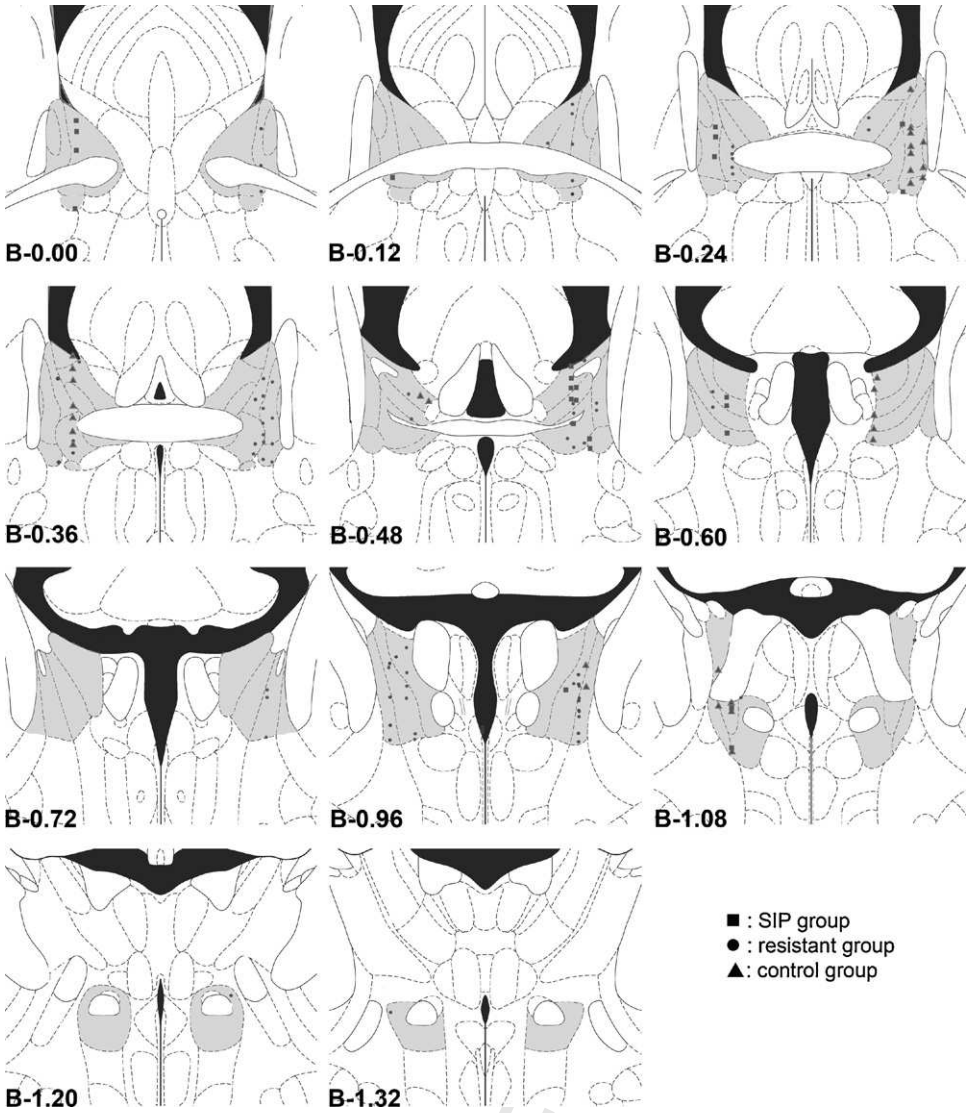


Fig. 1. Localization of the recording positions in the schedule-induced polydipsia group (■), the control group (▲), and the resistant group (●). The bed nucleus of the stria terminalis is colored in gray. Distance of the coronal sections posterior to bregma is indicated. Adapted from [16].

three groups (generalized estimating equations modeling, $p = 0.25$, 0.50, and 0.91, respectively). Two examples of recordings are shown in Fig. 2 for illustration.

3.1. Objective 1: firing rate and firing pattern in SIP rats, resistant rats and control rats

The firing rates and firing pattern parameters of the BST neurons of the different experimental groups are shown in Fig. 3. The firing rate of BST neurons in SIP rats was significantly higher than that of BST neurons in resistant rats (9.89 ± 1.99 vs. 5.32 ± 0.58, mean $\pm$ SEM, $p = 0.03$), but not in control rats (6.95 ± 1.02, $p = 0.44$). The variability of BST neurons in SIP rats was significantly lower than that of BST neurons in resistant and control rats (0.82 ± 0.05 vs. 1.29 ± 0.05 and 1.38 ± 0.10 respectively, $p < 0.001$ and $p = 0.002$). The burst index of BST neurons in SIP rats was significantly lower than that of BST neurons in resistant rats (0.04 ± 0.01 vs. 0.084 ± 0.02, $p = 0.049$) but not in control rats (0.082 ± 0.01, $p = 0.26$). The randomness of BST neurons in SIP rats was significantly higher than that of BST neurons in resistant and control rats (0.42 ± 0.03 vs. 0.02 ± 0.05 and 0.09 ± 0.04, respectively, $p < 0.001$ and $p < 0.001$).

To investigate whether the obtained results are specific only for BST neurons, we repeated the analysis using the data from neurons that were not located in the BST. A total of 365 neurons from neighboring structures were recorded, with 46 neurons from the SIP group ($n = 7$ rats), 201 neurons from the RES group ($n = 26$ rats), and 118 neurons from the CON group ($n = 10$ rats). There was no difference in the position (subdural) of the recording sites between the three groups (generalized estimating equations modeling, $p = 0.63$).

It was found that the higher firing rate of SIP rats vs. resistant rats was specific for the BST neurons, since for the non-BST neurons, the firing rate was lower for the SIP rats compared the control rats and borderline significantly lower compared to resistant rats (3.36 ± 0.73 vs. 6.28 ± 0.61 and 4.80 ± 0.42 respectively, $p = 0.004$ and $p = 0.054$). The higher randomness in SIP rats compared to the resistant or control rats seems not only to be present in the BST, but also in the neighboring structures (0.16 ± 0.05 vs. -0.07 ± 0.04 and -0.09 ± 0.06, respectively, $p = 0.03$ and $p = 0.02$). There were no

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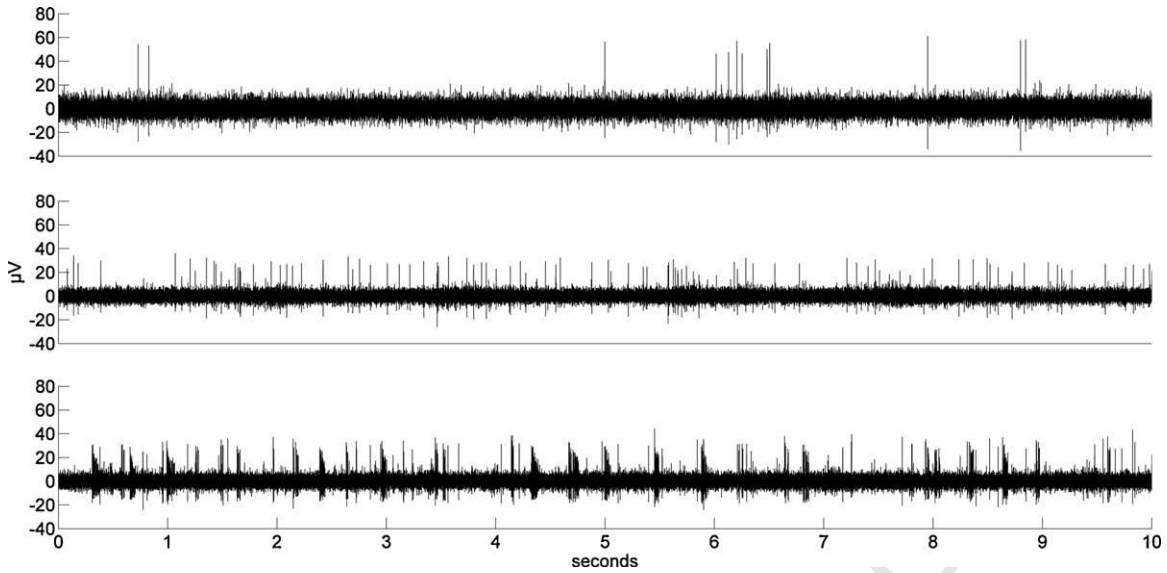


Fig. 2. Example of a recording of 10 s of one neuron in three animals. Upper panel: a neuronal recording of a control rat with a low firing rate (1.5 Hz), high variability (5.79), a low burst index (0), and low randomness (−0.47). Middle panel: a neuronal recording of a polydiptic rat with a medium firing rate (4.5 Hz), a medium variability (1.13), a medium burst index (0.1) and a medium randomness (0.23). Lower panel: a neuronal recording of a resistant rat with a high firing rate (20.2 Hz), medium variability (1.88), a high burst index (1.6), and low randomness (−1.65).

significant differences in variability or burst index between the groups in the non-BST neurons.

3.2. Objective 2: left-right hemisphere comparisons

In the SIP group, BST neurons from the right hemisphere showed a higher burst index than neurons from the left hemisphere (0.07 ± 0.02 ($n = 12$) vs. 0.03 ± 0.01 ($n = 17$), $p = 0.04$) (see Fig. 4). However, in the resistant and control group, neurons from the left hemisphere showed a higher burst index than neurons from the right hemisphere (0.06 ± 0.01 ($n = 47$) vs. 0.11 ± 0.04 ($n = 67$), $p = 0.007$ and 0.10 ± 0.02 ($n = 25$) vs. 0.07 ± 0.02 ($n = 28$), $p = 0.03$). Also, in the control group, there was a significant difference in variability between neurons from the left and right hemisphere (1.63 ± 0.18 ($n = 25$) vs. 1.17 ± 0.06 ($n = 28$), $p = 0.02$). This difference in variability is not present in the SIP (left 0.79 ± 0.07 vs. right 0.86 ± 0.08, $p = 0.71$) or resistant group (left 1.45 ± 0.10 vs. right 1.18 ± 0.05, $p = 0.20$). There were no differences observed in firing rate or randomness between hemispheres.

In addition, when focusing only the left or right hemisphere and performing the group analysis again (see Table 1), we may conclude that the lower burst index in SIP rats may be more attributed to the left than the right hemisphere. The lower variability and the higher randomness seemed to be present in the left as well as in the right hemisphere. The higher overall firing rate was not present in the left or the right hemisphere alone but combining data from both hemispheres, and thus increasing statistical power, may have caused the difference to become significant.

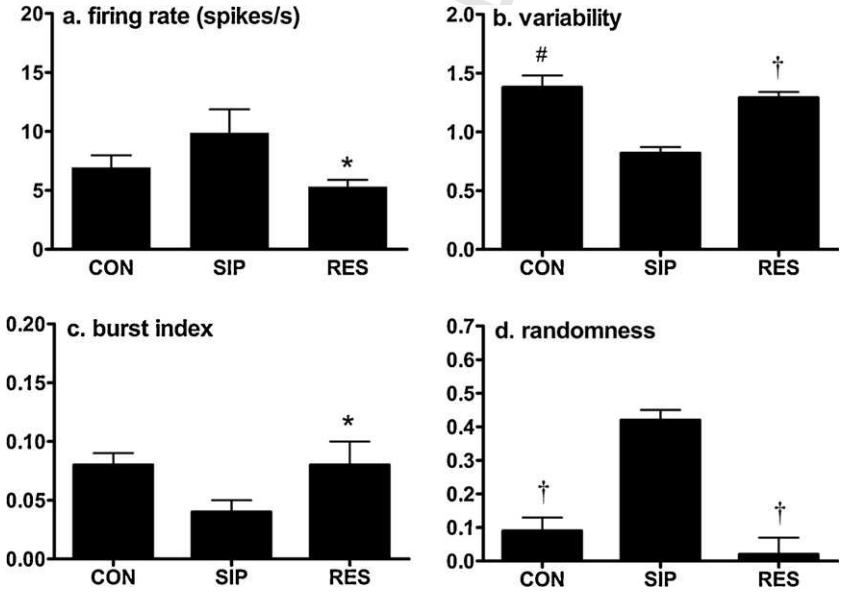


Fig. 3. Mean (±standard error of the mean) firing rate (a), variability (b), burst index (c), and randomness (d) in all groups. CON, control; RES, resistant; and SIP, schedule-induced polydipsia. *Significantly different from the SIP group at $p < 0.05$. #Significantly different from the SIP group at $p < 0.01$. †Significantly different from the SIP group at $p < 0.001$.

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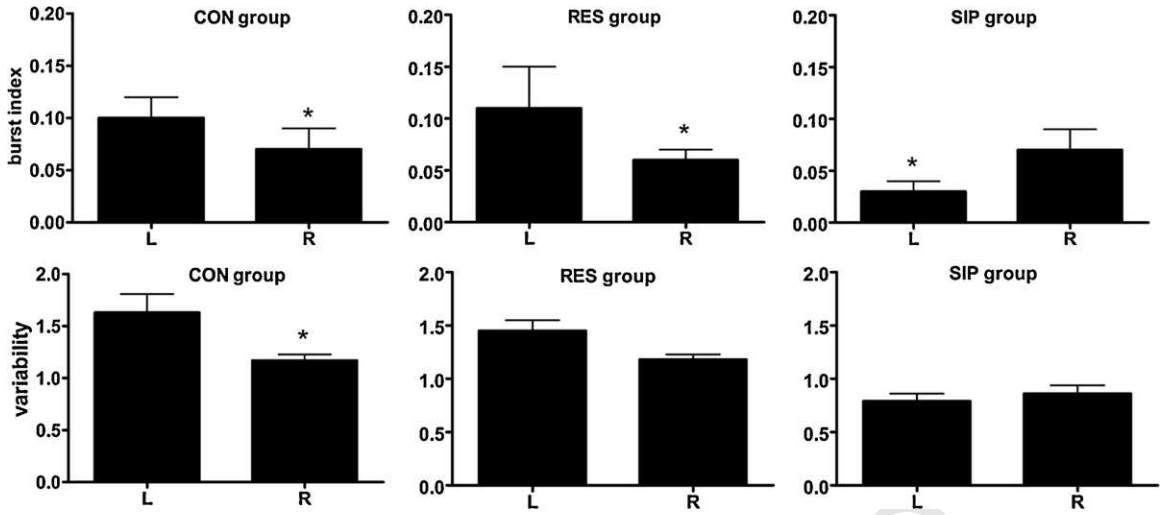


Fig. 4. Mean (±standard error of the mean) burst index (top row) and variability (bottom row) for the left and right hemisphere per group. CON, control; RES, resistant; and SIP, schedule-induced polydipsia. *Significantly different from the other hemisphere at $p < 0.05$. #Significantly different from the other hemisphere at $p < 0.01$.

Table 1
Group comparisons in the hemisphere-stratified analysis and the overall analysis in firing rate and firing pattern parameters.

		LEFT Mean ± SEM	RIGHT Mean ± SEM	Overall Mean ± SEM
Firing rate	SIP	11.34 ± 3.11	7.84 ± 1.94	9.89 ± 1.99
	RES	5.80 ± 1.06	4.99 ± 0.64	5.32 ± 0.58*
	CON	6.99 ± 1.10	6.93 ± 1.68	6.95 ± 1.02
Variability	SIP	0.79 ± 0.07	0.86 ± 0.08	0.82 ± 0.05
	RES	1.45 ± 0.10***	1.18 ± 0.05^	1.29 ± 0.05***
	CON	1.63 ± 0.18***	1.17 ± 0.06^	1.38 ± 0.10**
Burst index	SIP	0.03 ± 0.01	0.07 ± 0.02	0.04 ± 0.01
	RES	0.11 ± 0.04***	0.06 ± 0.01	0.084 ± 0.02*
	CON	0.10 ± 0.02***	0.07 ± 0.02	0.082 ± 0.01
Randomness	SIP	0.43 ± 0.03	0.41 ± 0.07	0.42 ± 0.03
	RES	-0.10 ± 0.08***	0.11 ± 0.05*^	0.02 ± 0.05***
	CON	-0.03 ± 0.06***	0.19 ± 0.05*^	0.09 ± 0.04***

Data are depicted as mean ± SEM.
* Significantly different from SIP group at $p < 0.05$.
** Significantly different from SIP group at $p < 0.01$.
*** Significantly different from SIP group at $p < 0.001$.
BST, bed nucleus of the stria terminalis; SIP, schedule-induced polydipsia group; RES, resistant group; and CON, control group.

Table 2
Group comparisons in the antero-postero-stratified analysis and the overall analysis in firing rate and firing pattern parameters.

		Anterior BST Mean ± SEM	Middle BST Mean ± SEM	Posterior BST Mean ± SEM	Overall Mean ± SEM
Firing rate	SIP	4.05 ± 1.10	13.10 ± 2.88	12.60 ± 7.34	9.89 ± 1.99
	RES	4.42 ± 0.87	5.20 ± 0.88***	5.89 ± 1.05	5.32 ± 0.58*
	CON	4.71 ± 1.82	8.09 ± 1.45*	6.28 ± 1.29	6.95 ± 1.02
Variability	SIP	0.98 ± 0.06	0.71 ± 0.08	0.80 ± 0.12	0.82 ± 0.05
	RES	1.34 ± 0.15*^	1.15 ± 0.04***	1.39 ± 0.08***	1.29 ± 0.05***
	CON	1.09 ± 0.05***	1.36 ± 0.06**	2.08 ± 0.64**	1.38 ± 0.10**
Burst index	SIP	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
	RES	0.06 ± 0.01	0.06 ± 0.01	0.12 ± 0.04***	0.084 ± 0.02*
	CON	0.06 ± 0.02	0.10 ± 0.02	0.04 ± 0.02***	0.082 ± 0.01
Randomness	SIP	0.36 ± 0.04	0.45 ± 0.05	0.47 ± 0.09	0.42 ± 0.03
	RES	0.08 ± 0.10***	0.11 ± 0.05***	-0.08 ± 0.08***	0.02 ± 0.05***
	CON	0.22 ± 0.06***	0.04 ± 0.05***	0.02 ± 0.13***	0.09 ± 0.04***

Data are depicted as mean ± SEM.
* Significantly different from SIP group at $p < 0.05$.
** Significantly different from SIP group at $p < 0.01$.
*** Significantly different from SIP group at $p < 0.001$.
BST, bed nucleus of the stria terminalis; SIP, schedule-induced polydipsia group; RES, resistant group; and CON, control group.

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3.3. Objective 3: correlations with position of the neurons within the BST

In the resistant group, a more lateral position of the recording resulted in a lower variability of the BST neurons, as well as a lower burst index ($p = 0.04$ and $p = 0.01$, respectively). When we compared all neurons from all groups, we observed a higher burst index with increasing (more ventral) depth of recording ($p = 0.02$). No other correlations were observed.

In addition, we performed an antero-postero-stratified analysis (anterior BST: B-0.00 to B-0.24, middle BST: B-0.36 to B-0.60 and posterior BST: B-0.72 to B-1.08). The results of this analysis are in accordance with the overall analysis (see Table 2). It may be concluded that the higher overall firing rate in SIP rats may be attributed to the middle part of the BST, and the lower overall burst index to the posterior part of the BST. The lower variability and the higher randomness seemed to be present in the anterior parts as well as in the middle and posterior parts of the BST.

4. Discussion

We investigated the single-cell activity of neurons in the bed nucleus of the stria terminalis in rats in an animal model for OCD compared to the activity in resistant and control rats. The study provides a summary of the BST firing properties under urethane anesthesia, which were not well described in the literature so far. Notably, we provide evidence for left-right differences in the firing patterns and a dorso-ventral gradient of the bursting index distribution.

4.1. Neuronal activity in the BST

Mean firing rates of control animals were similar to the values already reported in literature. Low firing rates are also reported by [26–29]. Other BST firing properties are not extensively described in literature, which makes comparison difficult. A common way of evaluating the firing pattern of neurons is to make a classification in regular, irregular, and bursting neurons based on the interspike interval histograms [30]. However, due to the low firing rates of neurons in the BST, this was not possible. Instead, the variability, the burst index, and the randomness were used as parameters to describe the firing pattern.

The variability of the neuronal firing in SIP rats was lower than in control or resistant rats, suggesting a more regular firing pattern in SIP rats. However, the randomness was higher, which is an indication of more irregular firing [30] and more true spontaneous activity (in the sense of neuronal activity that is not related in any obvious way to external stimulation [31]). Low signal-to-noise ratio, and thus errors in clustering, can occasionally affect some of the parameters extracted from interspike interval histograms, which are used in this study. It has been suggested that in this case, variability shows more robustness than, for instance, mean firing frequency [18]. Hence, in the interpretation of such ambiguous data, one may put more emphasis and weight on the variability parameter than on the randomness parameter.

This combination of low variability and high randomness is not a new observation. It was also found in olfactory receptor neurons in tracheotomized rats compared to freely breathing rats [32]. The tracheotomy was done to minimize the possible external effects/stimuli that might change the properties of the spontaneous activity.

Regarding firing rate and bursting behavior (respectively, higher and lower in SIP rats), there is only a significant difference between SIP rats and resistant rats, and not control rats. The decreased bursting behavior in SIP rats compared to resistant rats also suggests a more regular firing pattern in SIP rats. Generally, spike trains with a variability < 1 appear more regular, while a variability > 1 is often used as a marker for bursting activity [23,32]. Indeed, we found less bursting behavior for the groups with mean variability < 1, i.e. the SIP group, compared to the control (not significant) and resistant (significant) group, which have a mean variability > 1.

The higher firing rate in SIP rats compared to resistant rats suggests that rats that are susceptible to the SIP model (and thus to developing obsessive–compulsive behavior) have a higher neuronal activity in the BST than rats that are resistant and not susceptible to the SIP model.

In some cases, unilateral deep brain stimulation is sufficient to relieve a patients' symptoms [14,15]. In this context, a comparison of the neuronal activity in the BST between hemispheres was made. However, no major differences were found. The bursting behavior in rats in the SIP group in the right hemisphere was larger than in the left hemisphere, and the opposite was true in the control or resistant group. To our knowledge, no such left-right differences in the BST were described in the literature so far.

The BST consists of many subnuclei (based on their projection pattern and neurochemical identity) that have different functions [33]. This may be the reason for the relatively large variability in the results obtained. However, it was not possible to make an analysis per subnucleus, due to the limited amount of data points in some subnuclei. This is why a correlation with the position of the neurons within the BST was sought. Recently it has been shown that neurons projecting from the BST to the ventral tegmental area, which mainly reside in the ventral BST, have lower capacitance, higher input resistance, inward rectifying potassium currents, and lack I_h currents [34,35]. These properties suggest that these cells may be more easily excited by synaptic input. The low threshold spike activity in the ventral BST may result in increased bursting phenomena [36]. In this study we found indeed a higher bursting index with increasing (more ventral) depth of recording.

4.2. The BST in relation to OCD

Currently, lesions and pharmacological manipulations of the BST suggest roles in the physiology of stress, anxiety, food intake, social behaviors, pain, goal-directed behaviors, anorexia, and addiction. Despite the findings of [7] and [13], the BST has not yet been directly linked to OCD, although anorexia is often comorbid in OCD patients. Furthermore, anxiety is a cardinal symptom in OCD, leading to compulsions even when there is no imminent threat (anymore). A link to stress can also be made; one of the most popular motivational hypotheses of schedule-induced polydipsia attributes this behavior to a coping process. The stress related to the scheduled delivery of food pellets in the hungry rats in the current model leads to the compulsive drinking, which is interpreted as a means of reducing the aversive feelings [37–39]. In a functional magnetic resonance imaging (fMRI) study of OCD symptom provocation, activation of the amygdala was observed [40]. The basolateral part of this nucleus is the major source of afferents to the BST, and the central part of the amygdala is anatomically, neurochemically, cytoarchitectonically, and embryonically related to the BST [41]. However, the BST is more involved in the processing of anxiety-induced stimuli in opposition to the amygdala, which are involved in the processing of fear-inducing stimuli [42,43].

Dysregulation of the serotonergic system has been implicated in the pathophysiology of OCD and has been suggested primarily on the basis of the effectiveness of serotonin reuptake inhibitors (SSRIs, e.g. fluoxetine, clomipramine) in alleviating obsessions and compulsions in patients, as well as in rats in the SIP model. The BST receives a fairly dense innervations by serotonergic afferents [44,45] and multiple serotonergic receptor subtypes are expressed within this region. The function of serotonin in the BST is to dampen

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the activity (and, consequently, anxiety-like behavior) during exposure to threatening stimuli. However, changes in the balance of the function of BST serotonin receptor subtypes could alter the response of BST neurons to favor excitation and produce a pathological state of anxiety [46]. Our observations of higher firing rates in BST neurons in rats in the schedule-induced polydipsia rats compared to resistant rats also imply a higher neuronal activity in the BST in this OCD model. Furthermore, the BST may be an important site of action in the pharmacological (or other) therapies in the treatment of anxiety disorders including OCD.

Although currently SSRIs are the most effective treatment for OCD, still one third of the patients do not respond to this medication [47]. The combination of atypical antipsychotics and SSRIs has been found to induce significantly larger improvement of symptoms in patients [48–50], but studies with dopamine agonist and dopamine reuptake inhibitors have shown exacerbation of OCD symptoms in some, but not all, patients with OCD [51]. Hence, besides serotonin also dopamine may be implicated in the pathophysiology of OCD. There is direct support for a role of dopamine in the pathophysiology of OCD from neuroimaging studies that show higher densities of the dopamine transporter along with a down regulation of the D2 receptors in the basal ganglia of OCD patients. Furthermore, an increased dopamine signaling in the cortico-striato-thalamic circuitry has been related to OCD [51–53]. As mentioned before, the BST has strong connections to this circuitry [8] and neuroimaging studies demonstrated the importance of this circuitry in the pathophysiology of OCD [9]. Also, The BST may process emotional and context-dependent stimuli and integrate these in the dopaminergic reward/motivation circuitry [54].

An emerging body of evidence supports the hypothesis that also a dysregulation of glutamate neurotransmission may contribute to the pathophysiology of OCD [55]. It has been suggested that a frontocortical hyperglutamatergic dysfunction underlies the cortico-striatal-thalamo-cortical abnormalities observed in imaging studies of OCD [9] and also animal models confirm the role of corticolimbic glutamatergic hyperactivation in patients with OCD [56]. In the BST, there does appear to be a distinct glutamatergic population of projection neurons as well as evidenced by functional assays and the presence of mRNA of multiple vesicular glutamate transporter genes [57] (Allen Institute for Brain Science, 2008). This population has been identified to play a key role in anxiety-like behaviors, under which OCD is classified [58].

4.3. General considerations

Although we started with a large group of rats that were trained in the SIP model, only few (21%) became polydiptic. A training period of 15 days on week days seemed appropriate [59,60]. However, adding more training days or extending the duration of the training sessions [10] may solve this problem in future experiments.

The BST (more specifically the center part) is sexually dimorphic in terms of its size and neurochemistry, both in humans [61] and in rats [62]. Because the prevalence of OCD in humans is the same for both sexes, the gender of the rats was chosen to be male in order to have no interference with the changes in energy metabolism that occur during the estrus cycle of female rats [63].

We performed neuronal recordings in animals under general anesthesia. Although we realize that the neuronal activity could differ from freely moving awake animals, it is a great challenge to do awake recordings in this animal model. The rats are highly excited and practical difficulties arise when trying to record neuronal activity (e.g. movement artifacts, loose connections). Thus, this study should be considered as a prerequisite for studies in freely moving animals, where neural activity could be correlated to OCD-like behavior (eating and drinking).

4.4. Conclusions

The presented findings suggest that rats in the schedule-induced polydipsia, which models compulsive behavior in humans, display changes in the firing behavior of BST neurons. Further research in freely moving animals and in imaging studies could give more insight in the neuronal circuitry of the obsessive–compulsive disorder and thus may be of great aid to optimize therapies for OCD patients.

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