SHORT COMMUNICATION

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Stimulus-seeking in rats is accompanied by increased c-Fos expression in hippocampal CA1 as well as short 22 kHz and flat 50 kHz calls

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We determined CA1 hippocampal field to be involved in self-exposure, a type of novelty-seeking behaviour that has also been associated with short 22 kHz and flat 50 kHz ultrasonic vocalizations (USV) in adult male Long-Evans rats. Rats were habituated for three days to a self-exposure cage with two nose-poke holes. On day four, the animals from the experimental group were allowed to turn the cage light off for 5 s with a nose-poke (test/self-exposure session), while rats from control-yoked group had changing light conditions coupled and identical to the experimental animals. The experimental rats performed more nose-pokes during self-exposure session than animals from the control group. This effect was accompanied by a higher density of c-Fos-positive nuclei in the hippocampal CA1. There were no significant group differences in c-Fos expression in other brain regions analysed. However, possible involvement of several other structures in self-exposure (i.e., CA3, the dentate gyrus, amygdala, prefrontal cortex, and nucleus accumbens) is also discussed, as their correlational activity, reflected by c-Fos immunoactivity, was observed in the experimental rats. During test sessions, there were more nose-pokes accompanied by short 22 kHz calls and 50 kHz calls performed by the rats of the experimental group than of the control group. The CA1 region has previously been associated with novelty; short 22 kHz USV and flat 50 kHz USV could be associated with self-exposure, also they appear to be emitted correlatively.

Key words: stimulus-seeking, self-exposure, instrumental learning, vocalizations, hippocampus

Voluntary exploration is a complex type of behaviour that involves emotional, motivational, and cognitive components and is therefore associated with significant and coordinated activation of numerous brain areas (Hess et al., 1995; Mohammed et al., 2002; Turner et al., 2002; Balleine 2005; Knapska et al., 2006; Bourgeois et al., 2012; Kinnavane et al., 2014; Larkin et al., 2014; Comba et al., 2015; Mun et al., 2015). We study one type of exploratory behaviour: 'stimulus-seeking' or 'self-exposure' behaviour. Our laboratory (Matysiak 1978; Farley and Matysiak, 2008; Osinski and Matysiak, 2008) has previously demonstrated that, when given the opportunity, rats have a tendency to control their environment by switching the light on/off *via* a nose-poke.

We looked for the parts of the rat brain activated during the self-exposure, namely: the basolateral (BLA) and central (CeA) amygdalar nuclei, pre- (PrL) and infralimbic (IL) cortices, shell (AcbSh) and core (AcbC) of the nucleus accumbens. We also examined CA1, CA3, and the dentate gyrus (DG) of the hippocampal formation, as their involvement in controlling and guiding emotions, motivation, and cognition is well documented (Hess et al., 1995; Mohammed et al., 2002; Turner et al., 2002; Knapska et al., 2007). As a method of choice, we used the c-Fos protein, which is a product of immediate-early genes and expression lev-

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els are often utilised as a marker of neuronal activity and plasticity, e.g. in our previous works (Kaminska et al., 1997; Jaworski et al., 1999; Filipkowski et al., 2000; 2001).

Ultrasonic vocalizations (USV) emitted by rats are regarded as indicators of their emotional state (Brudzynski 2015). There are at least 3 types of USV in adult rats (Portfors, 2007; Brudzynski, 2013): 50 kHz USV, indicating positive emotional states; long 22 kHz calls (>300 ms), signalling an aversive state, and short 22 kHz calls (<300 ms), whose function is still ambiguous; however, they are usually also regarded as expressing aversion-like, negative emotional states (Barker et al., 2015; Brudzynski, 2015). Short 22 kHz USV were frequently observed in rats with low, sub-satiety doses of self-administered cocaine (Barker et al., 2010; 2014). They were also observed in several aversive experimental situations (listed in Barker et al., 2015). In general, they were not observed in non-aversive setups. Here, we studied emission patterns of different USV during the stimulus-seeking procedure.

Twenty male Long-Evans rats, 4 months old, were housed in pairs, with natural light/dark cycle, and water and food provided *ad libitum*. All procedures were approved by Local Ethics Committee for Animal Experimentation. Stimulus-seeking/self-exposure was performed in 33 × 30 × 27 cm chambers made of plexiglas (back and front) with side aluminium walls equipped with a lightbulb (30 lx). The chamber floors were made of metal bars, 0.5 cm in diameter, mounted every 1.7 cm. In each chamber, the right side of the aluminium wall had two circular holes, 3 cm in diameter, 14 cm apart, 2 cm above floor level, with photocells to register and count nose-pokes as radius interruptions. Each chamber was inside a noise-attenuating wooden box (64 × 38 × 60 cm), equipped with a fan for proper air circulation. The apparatus was controlled and monitored by computer software (PC-Med Med Associates Inc.).

The behavioural procedure started with handling; all rats were handled for 3 min per day for 10 days to habituate them with the experimenter. This was followed by 3 habituation sessions wherein rats were placed individually in the self-exposure chamber for 30 min per day for 3 consecutive days. The following day, during the test/self-exposure session, the rats were assigned to two groups and placed again in the self-exposure chamber for 30 min. For experimental rats (n=10), activation of any photocell, performed during light-on, shut down the light for 5 s. Yoked (control, n=10) animals, in contrast, were unable to switch off the light. However, the illumination changed accordingly and paralleled the one in a coupled experimental cage; i.e., control rats experienced the same light conditions as their individually coupled experimental subjects. During all habituation sessions and the test session, the number of nose-pokes was registered and USV were recorded with a high sensitivity condenser microphone (Avisoft Bioacustics), automatically detected and scored on the spectrogram with Avisoft SASLab Pro software (Avisoft Bioacustics). Three sub-types of USV were analysed: high frequency 50 kHz USV (32-96 kHz), long low frequency 22 kHz USV (18-32 kHz, 300-4000 ms (Portfors 2007)), and short low frequency 22 kHz USV (18-32 kHz, <300 ms (Brudzynski 2013)). The final USV classification was made by visual inspection of spectrogram images according to Wright et al. (2010) categorization.

For c-Fos immunocytochemistry, an hour and a half after the test, the rats were sacrificed with Morbital (Biowet Pulawy; i.e., sodium pentobarbital, 133.3 mg/ml, and pentobarbital, 26.7 mg/ml; 0.1 ml/100 g body weight, i.p.), perfused intracardially with 200 ml of ice-cold PBS followed by 200 ml of ice-cold 4% paraformaldehyde. The brains were removed and stored in the same fixative overnight at 4°C, and then stored in 30% sucrose with 0.02% sodium azide at 4°C. The brains were frozen instantly in dry ice-cold Heptane (Chempur) and coronal 45-µm-thick cryostat sections were collected at -20°C. For each structure analysed, there were 3 slices per rat selected, 45 µm thick, 2.04 - 3.00 mm posterior to bregma for the amygdala and hippocampal formation, and 3 different slices, 2.52 - 3.24 mm anterior to bregma for both the pre- and infralimbic cortices as well as the nucleus accumbens (Paxinos and Watson, 2007). The sections were washed three times in phosphate-buffered saline (PBS), incubated for 10 min in 0.3% H_2O_2 solution (Sigma), washed in PBS, and incubated for 48 h with primary c-Fos antibody (1:1000, no. sc-52, Santa Cruz) with 3% normal goat serum (NGS, Vector Laboratories) at 4°C. Then the slices were washed three times in PBS with 0.3% Triton-X 100 (Sigma) and the secondary antibody incubation was performed (1:1000, goat biotinylated, Vector Laboratories) in PBS/Triton with 3% NGS for 4 h, followed by PBS/Triton washes and avidin-biotin peroxidase complex incubation (1:100, 1:100, no. PK-6100, Vector Laboratories) in PBS/Triton for 1 h. The sections were then washed three times in PBS and stained with diaminobenzidine (Sigma) in water. When the expected level of staining was achieved, the slices were washed with PBS. Slices were mounted on gelatin-covered slides, dehydrated in gradually increasing ethyl alcohol dilutions and xylenes (Chempur) and embedded in Depex (Serva). Nissl staining; selected, adjacent slices, were mounted on a gelatin-coated slides, dried, submerged in PBS followed subsequently by 70%, 100% and 70% ethyl alcohol solutions, 1 min each, water, then 30 s incubation in 0.5% Cresyl violet solution with acetic acid (0.35 M) and sodium acetate (0.06 M), and finally washed in water and dehydrated in ethanol, 70% and 100%, ethanol/xylenes (1:1), xy-

lenes and embedded in DePeX (Serva). For c-Fos-positive nuclei counting, the nuclei were marked, selected and counted according to their staining intensity with ImageJ software. The measure of c-Fos immunostaining was expressed as density of c-Fos+ nuclei in a given structure, i.e., the number of c-Fos-positive nuclei per 1 mm^2. The borders of investigated structures were determined with the use of the Nissl-stained sections. The nuclei within the borders were counted with the researchers being blind to the treatment.

Statistical analyses were performed (STATISTICA 7.1., Stat-Soft) using analysis of variance (ANOVA), and data are represented as mean with standard error of the mean (SEM), and with $p<0.05$ as the minimal level of significance. Significant ANOVA results were followed with Duncan *post hoc* tests. When assumptions for ANOVA were not met, data were Box-Cox transformed (JMP3.2.6; SAS Institute). The Mann-Whitney U test was used for independent groups, while the Friedman test was used for dependent groups when the transformed data still violated assumptions for ANOVA. Between-variable correlations were measured with nonparametric Spearman's correlation (rho, p) or parametric Pearson's correlation (r).

We found that rats performed more nose-pokes when nose-poking led to an alteration of the lighting conditions within the cage (Fig. 1A). For nose-poke numbers, there was a group effect ($F_{(1,18)}=4.61$, $p<0.05$), session effect ($F_{(3,54)}=4.82$, $p<0.01$), and session x group effect ($F_{(3,54)}=5.73$, $p<0.01$, repeated measures ANOVA). During

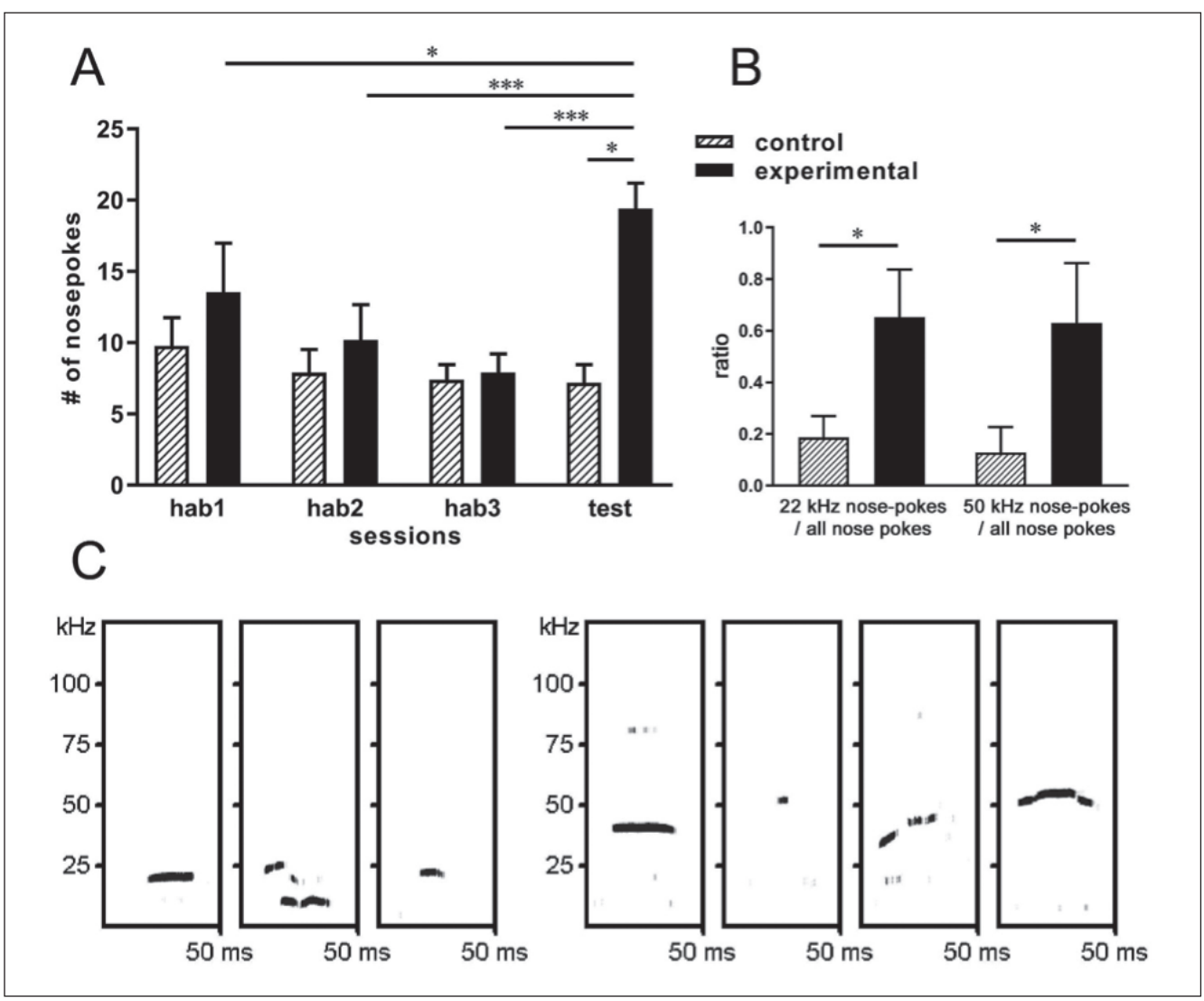

Fig. 1. (A) Mean (+SEM) number of nose-pokes during habituation sessions and the test session when only the experimental rats could turn the light off for 5 s with a nose-poke. (B) Ratio of nose-pokes associated with USV *vs.* all nose-pokes during the test session. *$p<0.05$, ***$p<0.001$. (C) Examples of ultrasonic vocalizations, from left to right: flat, multi-step, and short 22 kHz calls; flat, short, upward ramp, and inverted-U 50 kHz calls.

the test session, the experimental rats performed more nose-pokes than the control animals (p<0.05), and the number of nose-pokes was higher than during habituation sessions 1 (p<0.05), 2 (p<0.001), and 3 (p<0.001, all Duncan). Also, when the nose-pokes from three habituation sessions only were jointly analysed for all animals, there were more nose-pokes during session 1 *vs.* 3 (Z=1.98, p<0.05, Wilcoxon). This was despite the fact that the ANOVA analysis showed no effects.

There was higher c-Fos-positive-nuclei density in the CA1 region of the experimental rats *vs.* control animals ($F_{(1,14)}$=5.26, p<0.05; Fig. 2), and no group difference in any other area analysed, including: CeA ($F_{(1,16)}$=0.38, p=0.54), BLA ($F_{(1,1}$5)=4.53, p=0.05), IL ($F_{(1,18)}$= 0.61, p=0.44), PrL ($F_{(1,18)}$=0.20, p=0.68), AcbC ($F_{(1,17)}$=0.09, p=0.92), AcbSh ($F_{(1,18)}$=1.00, p=0.33), CA3 ($F_{(1,14)}$=0.83, p=0.37), and DG ($F_{(1,14)}$=2.69, p=0.12; all one-way ANOVA).

Regarding recorded USV, there were no long 22 kHz USV detected. However, short 22 kHz (830 cases) and 50 kHz USV (897 cases) were recorded. Among those, different types of USV were observed, for e.g. flat, multi-step, and short, as illustrated in Fig. 1C. Flat calls were predominant, for both 50 kHz (38%) and short 22 kHz USV (39.9%). Multi-step calls were the second

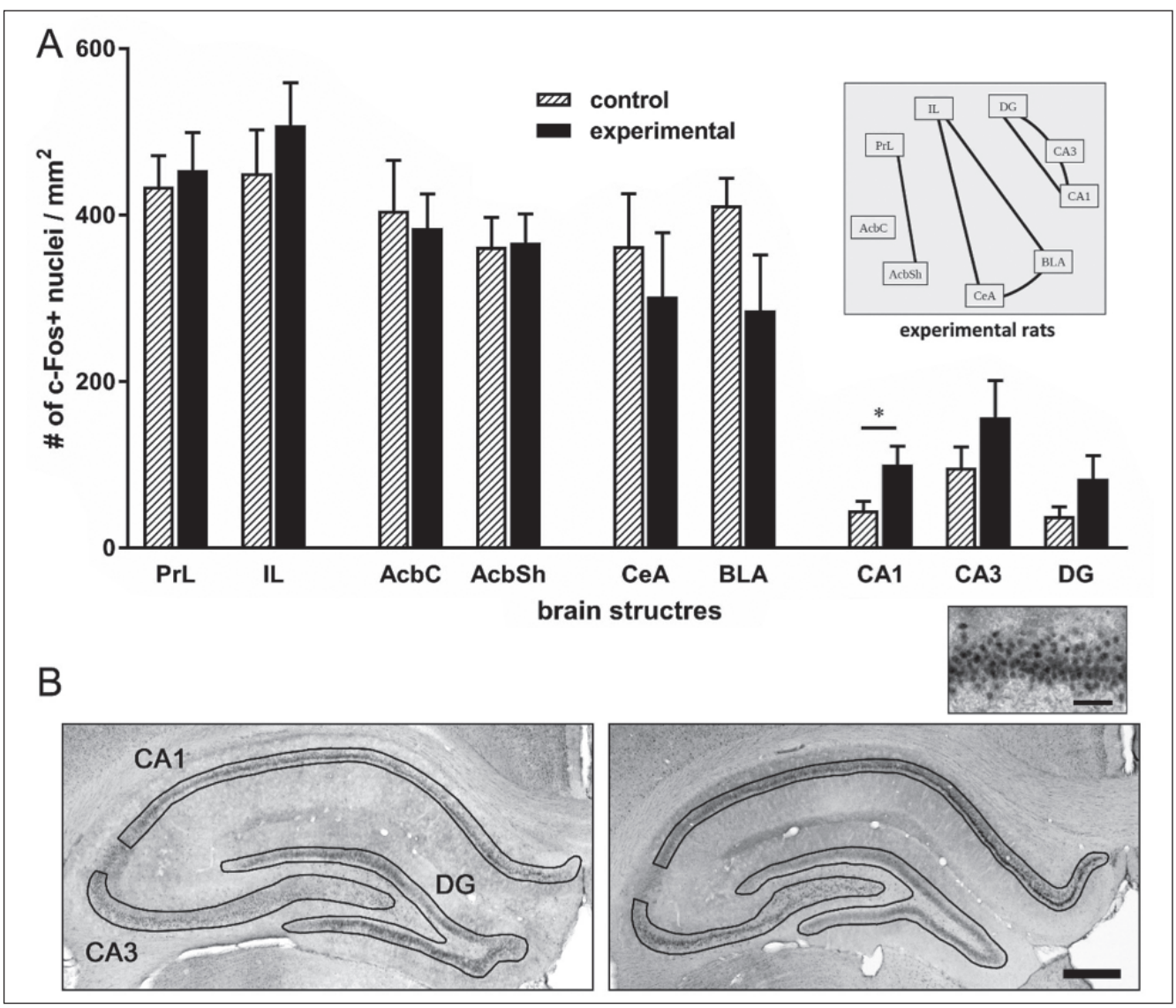

Fig. 2. (A) Mean (+SEM) number of c-Fos-positive nuclei per mm^2 in various brain structures, *p<0.05. Upper panel: network graph with significant positive correlations of c-Fos expression in the structures examined, in the experimental group (Spearman's ρ≥0.78, p<0.05). No significant correlations were found in the control group; BLA, the basolateral nucleus of the amygdala; CeA, the central nucleus of the amygdala; PrL the prelimbic cortex; IL, the infralimbic cortex; AcbSh, the shell of the nucleus accumbens; AcbC, the core of the nucleus accumbens. (B) Representative examples of c-Fos immunostaining of control (left) and experimental (right) rats, with CA1, CA3, and DG areas marked for analysis, bar=500 μm, and a section of CA1 field (upper panel, bar=50 μm).

most prevalent among short 22 kHz USV (22.6%), while multi-step (14.4%) and short USV (8.7%) were common within 50 kHz USV. Upward ramp, step up, step down, downward ramp, and inverted-U calls were present sporadically within both USV types.

For a number of short 22 kHz calls, there was no significant effect of session ($F_{(3,54)}$= 0.48, p=0.69, ANOVA) nor any group difference during habituation sessions 1 (U=36.5, p= 0.30), 2 (U=44, p=0.64), or 3 (U=30.5, p=0.13), nor during the test session (U=33, p=0.19, all Mann-Whitney U tests). Similarly, for a number of 50 kHz USV, there was no effect of session ($F_{(3,54)}$=0.18, p=0.91) and no group effects ($F_{(1,18)}$=0.94, p=0.34, ANOVA). However, when the number of nose-pokes that were accompanied by short 22 kHz USV (i.e., with the presence of ≥1 call within ±30 s from a nose-poke) was analysed for the test session, there was a difference between the groups (U=24, p<0.05, Mann-Whitney U test). Of note, only up to one nose-poke was considered for a given USV call. Rats from the experimental group had significantly more (4.1±1.2) such nose-pokes than the control animals (0.9±0.3). Similarly, there was a difference between the groups regarding the number of nose-pokes accompanied by 50 kHz USV during test session (U=24.5, p<0.05, Mann-Whitney U test). Rats from the experimental group performed more nose-pokes (3.8±1.2) than the controls (0.6±0.4). Importantly, there was a higher proportion of USV associated with nose-pokes *vs.* all calls during the test session, emitted by the experimental rats (0.7±0.2; 0.6±0.2) than the control ones (0.2±0.1, U=20, p<0.05; 0.1±0.1, U=22.50, p<0.05; Mann-Whitney U tests; Fig. 1C) for both 22 and 50 kHz USV, respectively. This difference was not observed for flat and multi-step short 22 kHz USV, nor for flat calls within the 50 kHz range. However, the nose-pokes performed during the last (i.e., test) session that were accompanied with flat 50 kHz calls correlated positively with the number of c-Fos-positive nuclei in the CA1 region of the hippocampus (r=0.50, p<0.05). This correlation was stronger in the experimental group (r=0.84, p<0.05) and was not observed in the control rats.

Finally, since short 22 kHz USV and flat 50 kHz calls seemed to follow a similar pattern and were both associated with the test-session nose-pokes, we verified that there actually was strong correlation between the number of short 22 kHz USV and the number of all 50 kHz USV (r=0.75, p<0.05) and flat 50 kHz USV (r=0.65, p<0.05) for all rats during all four days. Also, the correlation between all 50 kHz and short 22 kHz USV was observed during the test session (r=0.69, p<0.05). The correlation between high-frequency calls and short low-frequency calls was observed by others during tickling experiments (Schwarting et al., 2007).

In our experiments, we explored the self-exposure paradigm, a remarkably simple and unique – though recently disregarded – model to investigate emotionality, motivation, and learning in rats. We showed that the ability to control experimental conditions increased the reaction ratio, since the rats, when allowed to turn off the light with a nose-poke, performed more nose-pokes than during the previous session as well as more often than the control animals.

The observed goal-directed behaviours were associated with increased c-Fos expression in hippocampal CA1. Moreover, the number of test-session nose-pokes accompanied with flat 50 kHz calls correlated positively with the concentration of c-Fos-positive nuclei in the CA1 of all animals and in the experimental group, in particular. Notably, our task has an alternation of the behavioural protocol within test session, which constitutes an element of novelty. The latter has been repeatedly associated with increased c-Fos protein and mRNA expression in the hippocampal formation (e.g. Handa et al., 1993; Albasser et al., 2010; Tanimizu et al., 2018), and CA1 in particular (Kerr et al., 1996; Jenkins et al., 2004; Bourgeois et al., 2012; Jaeger et al., 2018). A specific involvement of CA1 in novelty-processing was also confirmed by an analysis of firing rate of place cells (Larkin et al., 2014; Zheng et al., 2016; Xu et al., 2019; Duvelle et al., 2019), shifting of preferred theta phase (Manns et al., 2007; Lever et al., 2010), coherence of theta/gamma-related CA1-input (Penley et al., 2013, Zheng et al., 2016), induced LTD (Manahan-Vaughan and Braunewell, 1999; Kemp et al., 2013), profound variations in population firing (Valenti et al., 2018), as well as, expression of other immediate-early genes (e.g. Hoang et al., 2018).

The degree of hippocampal involvement has been reported to vary depending on the type of novelty (Eichenbaum et al., 2007; Winters et al., 2008). Similarly, selective CA1 induction of c-Fos expression was repeatedly associated with specific characteristics of novelty, including: environmental novelty (rather than environmental complexity, Van Elzakker et al., 2008), novel spatial arrangements of familiar objects (rather than exposure to novel objects, Zhu et al., 1995; 1996; Wan et al., 1999), and a modified version of the familiar environment (*vs.* exposure to a completely novel environment, Sheth et al., 2008).

Moreover, novelty, with its potential environmental significance, leads to memory formation. It was shown that, following repetitive learning, added novelty can lead to improved performance and hippocampal c-Fos expression (Nikolaev et al., 1992), as discussed in Jaworski et al. (2018). Also, it was shown that separate prospective and retrospective modes exist in CA1; in particular, switching between the modes can possibly

prevent an interference between memory retrieval and encoding (Bieri et al., 2014). Similarly, hippocampal c-Fos-induction by triggering recognition memory occurs either in case of visual associative recognition (i.e., familiar items are reconfigured in a novel manner) or, as an exception, in the case of item recognition, but only when rats actively explore novel objects (Aggleton et al., 2012). The selective increase in c-Fos expression within CA1 subfield observed in our experiments could therefore be a result of a novel functional arrangement or reconfiguration of the nose-poke holes (i.e., added novel significance of a familiar object), which was actively explored and learned by the animals.

There was no difference in nuclear c-Fos expression between control and experimental groups in other investigated structures. This could be due to a very subtle type of stimulation, i.e., self-exposure, wherein the effects were compared to those of evoked stimulation experienced by control animals in the yoked group. It could also signify a crucial involvement of the CA1 field in this paradigm. However, several correlations of c-Fos expression in structures investigated were observed. Interestingly, statistically significant correlations at strong and very strong levels were observed only in the experimental group. These correlations (Spearman's rho coefficient, Fig. 2A), i.e., CA1 and CA3 (p=0.88), CA3 and DG (p=0.85), CA1 and DG (p=0.78), CeA and BLA (p=0.92), PrL and AcbSh (p=0.81), CeA and IL (p=0.80), as well as BLA and IL (p=0.80), might reflect an involvement of coordinated activity of those structures in the investigated behaviours. Several of these pairs include substructures of bigger anatomical formations, i.e., the amygdala (CeA and BLA) and the hippocampus (CA1, CA3, and DG), and their coordinated activation might reflect the overall contribution of a given structure in the self-exposure paradigm involving memory function (Squire 1992) and spatial representation (Moser et al., 2008), as well as, processing of emotions and storing the emotional aspects of memories (Knapska et al., 2007), respectively.

Other correlations involved the prefrontal cortex (PFC) which includes PrL and IL and exerts control of sensory and affective behaviours by active projections to other cortical and subcortical regions. Some PFC projections target the nucleus accumbens, a central node of reward circuitry, which is known for translating motivation into action (Klawonn and Malenka, 2019) and plays a role in several brain functions, including pain responses (Zhou et al., 2018), reward (Balleine 2005, Klawonn and Malenka, 2019), instrumental learning (Britt et al., 2012), and decision making (Walton et al., 2002).

Historically, intensely studied connections between the PFC and nucleus accumbens linked mainly PrL and AcbC. This circuit has shown to be involved in strategy switching (Cui et al., 2018), drug-seeking (Stefanik et al., 2016), social behaviours (Murugan et al., 2017) and, foremost, pain sensitivity (Lee et al., 2015; Martinez et al., 2017; Zhou et al., 2018). However, PrL exhibits dense direct projections to the AcbSh as well (Sesack et al., 1989; Ding et al., 2001; Vertes, 2004; Gabbott et al., 2005). These connections have been shown to be a part of a distinct circuit that also involves the medial ventral pallidum and the medial subdivision of the mediodorsal nucleus (O'Donnell et al., 1997; Groenewegen et al., 1999; Vertes, 2004). A role of this circuit in cognition, especially in working memory, has been postulated (Vertes, 2004), with PrL largely involved in the formation of response-outcome associations and reward prediction (Hart et al., 2014, Klawonn and Malenka, 2019) and AcbSh modulating risk-based decision making during instrumental learning (Stopper and Floresco, 2011). Optogenetic stimulations of AcbSh has also been shown to reinforce instrumental behaviour in mice (Britt et al., 2012). Finally, neural activity in AcbSh has been proposed to be particularly sensitive to conditions involving cognitive processing related to novelty (Stopper and Floresco, 2011).

The other part of the PFC, IL, appears to be functionally connected with two parts of the amygdala, i.e., CeA and BLA, during the self-exposure task. The amygdala is the key forebrain structure mediating inborn and acquired emotional responses, as well as, processing, interpreting, and integrating various aspects of biologically and/or emotionally relevant information (Knapska et al., 2007). There are direct IL–CeA, IL–BLA connections (Vertes, 2004; Knapska et al., 2007). In general, elevated PFC activity is correlated with decreased amygdala activity during tasks that require cognitive appraisal or regulation of emotion (summarised in Selleck et al., 2018), which was shown for both PrL and IL impacting BLA most likely through inhibitory intermediates, such as GABAergic interneurons. Further, the most prominent target of PrL and IL axons appears to be the BLA's anterior basal nucleus, wherein stimulation in either pathway evokes monosynaptic excitation and feedforward inhibition in BLA principal neurons (Arruda-Carvalho and Clem, 2014; 2015). It is postulated that this restrictive relationship between PFC and BLA activity can guide or regulate the expression of anxiety and fear (Selleck et al., 2018). However, others argue that the basomedial amygdala (BMA), involved in differentiating safe and aversive environments, represents the major target of PFC, especially IL, and mediates control of anxiety and fear (Adhikari et al., 2015). Interestingly, prominent BMA innervation was also visible in IL (Adhikari et al., 2015). Also, IL sends projections to CeA (Vertes, 2004). Of note, the amygdalar basolateral group is composed of the lateral, basal (together forming BLA), and BMA nuclei. This group is characterised by not only substantial interconnections with the neocortex but also within the group (Petrovich et al.,

1996; Knapska et al., 2007). In conclusion, in the self-exposure paradigm, there may be some cortical inhibition of amygdala-driven emotional reactions, i.e., inhibition of amygdalar activity by the cortex.

Finally, we show a connection between stimulus-seeking and the emission of both flat 50 kHz and short 22 kHz USV. The function of the latter is still not determined. It is generally accepted that rats emit USV in the 22 kHz range in response to negative stimuli (Brudzynski et al., 1993; 2007; Portfors, 2007; Kromkhun et al., 2013; Simola, 2015), see however Bialy et al. (2016). These USV can be further divided into long calls to signal external danger, and short ones that were proposed to express a state of discomfort without external danger (Brudzynski 2015). However, rats that self-administered binges of cocaine (Barker et al., 2010) and experienced tickling (Schwarting et al., 2007) were observed to emit short 22 kHz USV, which is not in agreement with the hypothesis of a strictly aversive role of the short calls. To the best of our knowledge, we are the first to observe this type of USV in a neutral behavioural context. Also, different types of frequency modulated 50 kHz USV have not been assigned with a clear behavioural function, although there is growing evidence of some possible assignments. For example, 50 kHz trills appear particularly in highly rewarding situations (Wright et al., 2012; Mulvihill and Brudzynski, 2018; Simola and Costa, 2018; Willadsen et al., 2018), while flat 50 kHz calls are recorded in more neutral settings (Burgdorf et al., 2008; 2011; Wohr et al., 2008), such as in our behavioural paradigm. Notably, novelty induces dopamine release in the hippocampus, triggering memory consolidation to boost memory persistence due to activation of dopamine receptors in CA1-CA3 and DG regions (Duszkiewicz et al., 2019). This can also manifest itself in the emission of typically reward-evoked USV.

In conclusion, active and voluntary switching off of lights by rats was accompanied by an increase in c-Fos expression in the hippocampal CA1 region and coordinated activity of several other structures. Together, these results indicate the complexity of the studied behaviour and suggest a key role of several elements as, among others, novelty, with a novel arrangement of familiar objects, (spatial) learning, regulation of emotional reaction, reward assessment, and USV emission.

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