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Anti-Streptococcus IgM Antibodies Induce Repetitive Stereotyped Movements: Cell Activation and Co-Localization with Fcα/μ Receptors in the Striatum and Motor Cortex

Danhui Zhang^a, Ankur Patel^{a,b,*}, Youhua Zhu^a, Allan Siegel^{a,b}, and Steven S. Zalcman^a

Ankur Patel: dr.ankur@yahoo.com

^aDepartment of Psychiatry, UMDNJ–New Jersey Medical School, Newark, NJ, United States

^bDepartment of Neurology & Neurosciences, UMDNJ–New Jersey Medical School, Newark, NJ, United States

Abstract

Group A beta-hemolytic streptococcus (GABHS) infections are implicated in neuropsychiatric disorders associated with an increased expression of repetitive stereotyped movements. Anti-streptococcus IgG presumably cross-reacts with elements on basal ganglia cells, modifies their function, and triggers symptoms. IgM may play a unique role in precipitating behavioral disturbances since variations in cortico-striatal activity occur in temporal congruity with peak IgM titers during an orchestrated immune response. We discovered in Balb/c mice that single subcutaneous injections of mouse monoclonal IgM antibodies to Streptococcus Group A bacteria induce marked dose-dependent increases in repetitive stereotyped movements, including head bobbing, sniffing, and intense grooming. Effects were antibody- and antigen-specific: anti-streptococcus IgG stimulated ambulatory activity and vertical activity but not these stereotypies, while anti-KLH IgM reduced activity. We suggest that anti-streptococcus IgM and IgG play unique roles in provoking GABHS-related behavioral disturbances. Paralleling its stereotypy-inducing effects, anti-streptococcus IgM stimulated Fos-like immunoreactivity in regions linked to cortico-striatal projections involved in motor control, including subregions of the caudate, nucleus accumbens, and motor cortex. This is the first evidence that anti-streptococcus IgM antibodies induce in vivo functional changes in these structures. Moreover, there was a striking similarity in the distributions of anti-streptococcus IgM deposits and Fos-like immunoreactivity in these regions. Of further importance, Fcα/μ receptors, which bind IgM, were present- and co-localized with anti-streptococcus IgM in these structures. We suggest that anti-streptococcus IgM-induced alterations of cell activity reflect local actions of IgM that involve Fcα/μ receptors. These findings support the use of anti-streptococcus monoclonal antibody administration in Balb/c mice to model GABHS-related behavioral disturbances and identify underlying mechanisms.

Keywords

Group A beta-hemolytic streptococcus; stereotypy; Obsessive–compulsive disorder; Tics; Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection

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*Corresponding author. Address: Department of Neurology & Neurosciences, UMDNJ–New Jersey Medical School, BHSB F 1532, 183 South Orange Avenue, Newark, NJ 07103, United States. Tel.: + 973 972 8161; fax: + 973 972 8305.

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1. Introduction

Group A beta-hemolytic streptococcus (GABHS) infections are implicated in neuropsychiatric disorders associated with an increased expression of repetitive behaviors, including motor stereotypies such as head shaking, sniffing, and touching movements (see Murphy and Pichichero, 2002; Church et al., 2003; Murphy et al., 2004, Murphy et al., 2010; Snider and Swedo, 2004; Leslie et al., 2008). Studies have shown that children suffering from pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), Tourette syndrome (TS), Sydenham's Chorea (SC), obsessive-compulsive disorder (OCD), and other tic disorders are twice as likely to have had a streptococcus infection preceding diagnosis (Mell et al., 2005; see also Leslie et al., 2008). Of further importance, anti-streptococcal antibody titers have been positively correlated with clinical severity in patients suffering from TS, autism spectrum disorder, and other disorders involving repetitive stereotyped movements (Vojdani et al., 2002). Tic disorders and other psychiatric disorders are often co-morbid; for example, about half of TS patients also display OCD (Albin and Mink, 2006).

Abnormalities in cortico-striato-thalamo-cortical (CSTC) circuits are thought to underlie symptom expression. In GABHS-related disorders, anti-neuronal antibodies are thought to cross-react with elements on cells within these circuits, particularly in the basal ganglia (Kiessling et al., 1993; Kirvan et al., 2006; Leckman et al., 2010). For example, increases in antibodies directed against elements in the caudate nucleus have been observed in patients suffering from SC or acute rheumatic fever (Husby et al., 1976; Church et al., 2002). These investigators further showed that antibody measures correlated positively with symptom expression. Antibodies against elements in the caudate nucleus and cortical motor regions were likewise found in a cohort of TS patients. Autoantibodies directed against elements in other brain regions (e.g., cortex and midbrain) have also been noted in patients (Verkerk et al., 2003). However, others have failed to observe any relationship between GABHS infections and anti-neuronal antibodies (see Harris and Singer, 2006). The extent to which such disparities are due to differences in patient populations, methodology, or other differences remains to be determined.

In view of the evidence linking anti-streptococcus antibodies with motor tics and altered basal ganglia function, investigators have determined whether microinjecting patient serum or its purified IgG fraction into rat striatal sites induces repetitive motor stereotypies. For example, it was demonstrated that infusion of sera from TS patients with high levels of antineuronal or antinuclear antibodies into the ventrolateral striatum induced oral stereotypies (Taylor et al., 2002). Intrastriatal microinfusion of TS sera or IgG similarly induced licking and head shaking in rats (Hallett et al., 2000). In contrast with such findings, however, microinjections of patient sera or antibodies directed against the streptococcal M5 protein into the ventrolateral striatum did not induce stereotypic behaviors (Loiselle et al., 2004). Moreover, increases in locomotion and vertical activity, including vertical stereotypic movements were induced in mice immunized and boosted with a GABHS homogenate or purified IgG from streptococcus infected mice (Hoffman et al., 2004; Yaddanapudi et al., 2009).

A common feature of these animal models is a focus on anti-streptococcus IgG. There is reason to suspect that IgM class antibodies may also play a role in precipitating behavioral disturbances since monoamine variations in CSTC motor circuits occur in temporal congruity with peak increases in IgM titers during an orchestrated immune response (Zalcman et al., 1991; Lacosta et al., 1994). For example, marked increases in dopamine release in the nucleus accumbens are evident around the time of the peak IgM response to

sheep red blood cells. Dopamine activity returns to baseline levels after the time of the peak IgM response (i.e., as IgM titers decline). In parallel, alterations in rates of responding for intracranial self-stimulation in mesostriatal sites are induced around the time of the peak IgM response (Zacharko et al., 1997). Of further relevance, peripheral injections of a monoclonal IgM antibody directed against amyloid beta protein penetrates the blood–brain barrier (BBB) and reverses cognitive deficits in mice (Banks et al., 2007). These investigators also showed that IgM accumulates in the brain within approximately 40-min of injection, and suggested that IgM would be expected to do so to a greater extent than IgG given the relative inefficiency of a saturable brain to blood efflux system. Since the IgM molecule is a pentamer, there would also be a greater potential for binding in the CNS. These findings coupled with that of Yaddanapudi et al. (2009) who showed that anti-streptococcus deposits are evident in the brain, raise the possibility that anti-streptococcus IgM may also accumulate in the brain, and play an important role in provoking GABHS-related behavioral disturbances and variations in cortico-striatal activity. Such effects may be expected to involve Fc receptors (FcR's) since antibodies bind to specific FcR's (see Okun et al., 2010). IgM class antibodies bind to Fc α/μ receptors (Fc α/μ R's) in a variety of tissues. Although there is a limited amount of information regarding their presence in the brain, Fc α/μ R's are expressed on oligodendrocytes and myelin in the subventricular zone of the lateral ventricle and the adjacent corpus callosum (Nakamura et al., 1993; Nakahara et al., 2003). It is not known whether Fc α/μ R's are expressed in CSTC motor circuits.

In the present study, we developed a model in which Balb/c mice receive single subcutaneous injections of mouse monoclonal IgM antibodies to streptococcus group A bacteria to determine whether: (1) anti-streptococcus IgM class antibodies induce tic-relevant repetitive stereotyped movements, and if so, whether potential effects are: (2) distinct from those induced by anti-streptococcus IgG, and (3) specific to the antigen against which IgM is produced. Given the links between anti-neuronal antibodies and CSTC circuits involved in motor control, we also sought to determine whether (4) anti-streptococcus IgM antibodies (a) accumulate- and (b) stimulate activity in subregions of the caudate, nucleus accumbens, and motor cortex. Of further importance, we also determined whether (5) Fc α/μ R's are present- and co-localize with anti-streptococcus IgM antibodies in these regions.

2. Materials and methods

2.1. Subjects

A total number of 74 male BALB/c mice, 2–3 months of age (mean weight of 24.96 ± 0.14 g) were used in the present study (6–9/group). The mice were obtained from Charles River Labs Inc. (Wilmington, MA), and housed in groups of four prior to being used as experimental subjects. The animals were maintained on a 12-h light/12-h dark cycle, and permitted ad libitum access to food and water. Experiments were conducted during the animal's light phase.

2.2. Anti-streptococcus and anti-KLH antibodies

Mouse streptococcus group A monoclonal IgM (Experiment 1) and IgG (IgG2a, Experiment 2) (abcam Inc., Cambridge, MA), and mouse keyhole limpet hemocyanin (KLH) monoclonal IgM (Experiment 3) (BD Biosciences, San Diego, CA) were dissolved in sterile saline, and injected subcutaneously (sc) at doses of 0.0, 6.25, or 12.5 μ g/mouse in a volume of approximately 0.20 ml. We used sc injections based on our unpublished experiments using anti-cytokine monoclonal antibodies, which in antibody-specific manners altered motor activity implying effects on CSTC circuits. In addition to anti-streptococcus IgM, we examined behavioral effects of anti-streptococcus IgG to determine whether anti-streptococcus antibodies induce class-specific behavioral effects. Anti-KLH IgM was used

to determine whether behavioral variations induced by anti-streptococcus IgM are a general property of IgM antibodies or whether they are specific to the antigen against which IgM is directed.

2.3. Behavioral Testing

Immediately following injections, the mice were individually placed into a test arena (TruScan Behavioral Monitoring System; Coulbourn Instruments, PA) in normal illumination for 2-h. A series of behavioral measurements were recorded including locomotion, vertical activity, horizontal and vertical stereotypic movements, jumps, and turns. Ambulation ('ambulatory distance') was defined as the sum of all vectored coordinate changes in the floor plane, and included floor plane total movement distance (cm) less the stereotypic movement distance. Vertical activity was defined as total movements in the vertical plane. Each movement is a series of successive coordinate changes with no rest (same coordinates) for at least 1 sample interval. Horizontal stereotypic movements were defined as the total number of coordinate changes ± 1.499 beam spaces in the floor plane (X and Y) dimension and back to the original point that do not exceed 2 s apart. Three such movements must be made before a stereotypy episode starts. When it does, the qualifying 3 movements are included in the total number of moves. Vertical stereotypic movements measures are analogous to those in the floor plan except that only vertical movements are measured. Turns were defined as movements where the animal enters 4 radially-contiguous quadrants in ascending or descending order, without interruption.

The test sessions were also filmed with a VHS camera, and at a later date, an experienced rater blinded to the treatment groups scored the incidence and duration of stereotypic behaviors, which included sniffing, head bobbing, and intense grooming, as per Kelley et al. (1988). We selected these stereotypies based on our unpublished data showing that they may be induced by elements of an activated immune system. The time engaged in head bobbing was defined as the time (s) engaged in repeated vertical head bobbing. A minimum of 3-s of uninterrupted head bobbing was defined as one episode. The duration of such episodes was recorded with a stopwatch. Sniffing was defined as the time (s) engaged in head-up and head-down sniffing with the head/snout directed towards the top of the cage or down toward the floor of the cage, respectively. We also measured the duration of such episodes as well as continuous sniffing for at least 5-s. Grooming was defined as grooming of the head or body as well as intense repetitive grooming of one body part. Measurements were taken determined 20-, 40-, 60-, 80-, 100- and 120- min after injection in 2-min epochs. Results are presented as totals ($\pm$ SEM) for the entire session. We also derived a stereotypy score by combining the percent increases in each of the observed stereotypies.

2.4. c-fos immunohistochemistry

Immediately following the test session of Experiment 1, the mice were deeply anesthetized with Na pentobarbital (60–80 mg/kg, IP) and transcardially perfused with 0.9% saline (pH 7.2) followed by 4% paraformaldehyde (pH 7.4). After perfusion, brains were removed from the skull and placed in 4% paraformaldehyde solution at 4 °C for overnight, and transferred to 30% sucrose solution at 4 °C until they sank to the bottom. The brains then were frozen in a cryostat (Leica CM1900) at –20 °C for slow freezing, and sections were cut at 30 μ m thickness and alternate sections were transferred to 24 well plates containing PBS, and washed for 30-min. Sections were then incubated on a rocker table with rabbit antibody to c-fos protein (Santa Cruz, CA) diluted in 1:500 in a PBS solution containing 1% normal goat serum and 0.3% Triton X-100 overnight at 4 °C. The next day, sections were washed with PBS and incubated in biotinylated goat anti-rabbit antibody (1:333, Vector Labs, Burlingame, CA) in PBS for 1 h. Sections are then rinsed in PBS and incubated for 90-min in avidin-biotin/peroxidase (ABC Elite, Vector Labs), after washed 3 $\times$ in PBS the sections

were treated with a ready-made stabilized solution of active diaminobenzidine ('Stable DAB', Invitrogen) until completion of the reaction (10 min), and were again washed, then dehydrated with alcohol, treated with xylene, and coverslipped with Permount (Sigma). Slides were viewed with an Olympus AX-70 microscope and photographed with a Magnafire digital camera. Images were taken at 4× magnification using Neurolucida software (Microbrightfield Inc., Williston, VT). Once the images were acquired, we defined the areas to be counted bilaterally as: (1) subterritories within the caudate nucleus, including the dorsomedial (DM), dorsolateral (DL), central (CT), ventromedial (VM), and ventrolateral (VL) aspects; (2) compartments of the nucleus accumbens, including the shell (sh) and core (co); (3) motor cortex, including M1 and M2 subdivisions. Particles that were counted were no smaller than 50 μm in area (to exclude background staining artifacts), and no larger than 130 μm (to eliminate large areas of immunoreactivity such as non-specific staining at the edge or at folds in the tissue). To distinguish between signal and background, sensitivity was set between 82% and 97%. Each Fos-positive nucleus was marked with an asterisk. Using Neuroexplorer 10 (Microbrightfield Inc., Williston, VT) software, we then counted the number of immunoreactive nuclei present within each of the pre-defined areas of different brain regions. Data from c-Fos immunohistochemistry (number of Fos-immunoreactive cells) were counted as the means of three sections (spaced 30 μm apart) per animal as described above.

2.5. Fluorescent labeling of anti-streptococcus IgM antibodies

Brain sections were first blocked with 5% normal goat serum containing 0.3% Triton X-100 in PBS for 1-h and then incubated with anti-streptococcus IgM monoclonal antibodies (mouse, 1:50 dilution) overnight at 4 °C followed by incubation with goat anti-mouse IgG-FITC (green, 518 nm, 1:200; Santa Cruz, CA) secondary antibody for 1-h at RT. For omission controls, the brain sections were only treated with goat anti-mouse IgG-FITC secondary antibody alone. Then the slices were mounted on a slide, and viewed under an Olympus fluorescence microscope.

2.6. Fluorescent labeling of Fc Receptors

Brain sections were first blocked with 5% normal goat serum containing 0.3% Triton X-100 in PBS for 1-h and then incubated with anti-streptococcus IgM monoclonal antibodies (mouse, 1:50 dilution) overnight at 4 °C followed by incubation with goat anti-mouse IgG-FITC (green, 518 nm, 1:200; Santa Cruz, CA) secondary antibody for 1-h at RT. Then the slices were mounted on a slide, and viewed under an Olympus fluorescence microscope. Brain sections were incubated with PE conjugated anti-CD351 (Fc α/μ receptor; BioLegend, Inc) antibody (red, 615 nm, 1:50 dilution; the solution is free of unconjugated PE and unconjugated antibody) overnight at 4 °C. Then the slices were mounted on a slide, and viewed under an Olympus fluorescence microscope.

For Fc γ receptors labeling, the brain sections were incubated with anti-CD16/32 (Fc γ II/III receptor) antibody (mouse, 1:50 dilution; BioLegend, Inc) overnight at 4 °C followed by incubation with goat anti-mouse IgG-FITC (green, 518 nm, 1:200; Santa Cruz, CA) secondary antibody for 1-h at RT. Then the slices were mounted on a slide, and viewed under an Olympus fluorescence microscope.

2.7. Statistics

Data were analyzed using a one-way or two-way (Antibody Treatment $\times$ Brain Subregion) analysis of variance (ANOVA) using GraphPad software. Bonferroni post-hoc tests ($\alpha = 0.05$) were used to confirm group differences.

3. Results

3.1. Single injections of anti-streptococcus IgM induce repetitive stereotyped movements

We developed a model to determine whether single injections of a mouse monoclonal IgM antibody to Streptococcus Group A bacteria induces repetitive motor stereotypies in Balb/c mice. We focused on IgM class antibodies because alterations in striatal activity and behavior are induced in close temporal congruity with peak IgM levels during an orchestrated immune response (Zalcman et al., 1991; Lacosta et al., 1994; Zacharko et al., 1997).

We discovered that single injections of anti-streptococcus IgM induce marked and dose-dependent increases in repetitive stereotyped movements, including head bobbing ($F(2,21) = 6.144, p < .01$), intense grooming ($F(2,21) = 5.79, p < .01$), and sniffing ($F(2,21) = 3.82, p < .05$). Post hoc comparisons revealed that compared with controls, the 6.25 and 12.5 doses of anti-streptococcus IgM induced significant increases in head bobbing (340% and 192%, respectively; Fig. 1A), intense grooming (71% and 164%, respectively; Fig. 1B), and sniffing behavior (58% and 33%, respectively; Fig. 1C). Intense grooming was significantly greater in mice receiving 12.5 μ g IgM compared to mice receiving the 6.25 dose.

Based on these findings, we derived a stereotypy score by combining the percent increases in each of the observed stereotypies. A significant change in the stereotypy score was induced by IgM treatment ($F(2,35) = 10.68, p < 0.001$; Fig. 1D). The 12.5 and 6.25 doses of IgM increased the stereotypy scores by 95% and 75%, respectively. Parenthetically, other doses of anti-streptococcus IgM tested did not produce behavioral changes of greater magnitude than the 6.25 μ g (dose data not shown). Peak effects of IgM on repetitive stereotyped movement were evident 40–100 min after IgM administration (Fig. 1E).

In contrast with these findings, anti-streptococcus IgM did not appreciably alter turning ($p = 0.20$), horizontal floor stereotypic movements ($p = 0.13$), rearing ($p = 0.78$), ambulatory distance ($p = 0.51$), or vertical stereotypic movements ($p = .24$); expressed as percent control (Table 1). The latter findings suggest that anti-streptococcus IgM do not reflect a general increase in activity.

3.2. Anti-streptococcus IgG stimulates vertical activity and locomotion

It is possible that the present effects of anti-streptococcus IgM antibodies were not specific to the antibody class per se. Thus, we repeated the previous experiment with one exception: the mice received monoclonal antibodies against anti-streptococcus IgG rather than IgM.

In contrast with anti-streptococcus IgM antibodies, anti-streptococcus IgG had no effect on head bobbing ($p = 0.69$), intense grooming ($p = 0.40$), or sniffing, $p = 0.33$) (Table 2). Turning and horizontal stereotypic movements were likewise unaffected by IgG treatment ($p > .90$; data not shown). However, vertical stereotypic movements ($p < 0.001$), rearing ($p < 0.05$) and locomotion ($p < 0.005$) (Table 2) were increased by anti-streptococcus IgG. Inasmuch as anti-streptococcus IgM and IgG induce unique behavioral profiles, we suggest that these anti-streptococcus antibody classes play unique roles in inducing behavioral disturbances following GABHS infections.

3.3. Anti-KLH IgM reduces activity

As discussed in the previous section, we found that the stereotypy-inducing effects of anti-streptococcus IgM are class-specific. However, it is also possible that these effects are characteristic of IgM class antibodies in general rather than being specific to anti-

streptococcus IgM per se. To address this possibility, we determined the effects of anti-KLH IgM on novelty-induced behavior.

In contrast with anti-streptococcus IgM, anti-KLH IgM induced a reduction in head bobbing ($p < 0.05$), rearing ($p < 0.05$) and sniffing ($p < 0.01$). Other behaviors, including vertical stereotypic movements, and ambulatory distance were unaffected by anti-KLH IgM, $p > 0.30$ (Table 3).

Based on these findings, we conclude that in addition to being class-specific, the stereotypy-inducing effects of anti-streptococcus IgM are antigen-specific. Of further importance, to our knowledge this is the first evidence that IgM class antibodies reduce activity.

Taken together, these experiments establish that anti-streptococcus monoclonal IgM antibodies induce marked increases in repetitive stereotyped movements, and that effects are dependent upon the class of anti-streptococcus antibody tested and the antigen against which IgM is directed. Thus, there is an exquisite specificity to the present monoclonal antibody-induced behavioral changes.

4. Single injections of anti-streptococcus IgM induce pronounced increases in Fos-like immunoreactivity in cortico-striatal regions

There is evidence that functional changes occur in cortico-striatal regions of patients suffering from TS and other tic disorders (Stern et al., 2000; Bohlhalter et al., 2006). Of further relevance, GABHS-related anti-neuronal antibodies have been found in CSTC circuits involved in motor control. However, there is no in vivo evidence that anti-streptococcus IgM induces functional changes in such structures. Because of this lack of evidence coupled with our finding that anti-streptococcus IgM induces tic-relevant behavioral changes, we determined whether anti-streptococcus IgM stimulates activity within the caudate, nucleus accumbens, and motor cortex.

4.1. Caudate nucleus

The number of Fos positive cells in the caudate nucleus varied as a significant interaction between Antibody Treatment and Brain Subregion ($F(4,54) = 9.82$, $p < 0.0001$; Fig. 2). Post-hoc comparisons confirmed that compared to their respective controls, IgM induced marked increases in Fos expression in the dorsomedial, ventromedial, dorsolateral, ventrolateral, and central aspects. The most pronounced increases were evident in the medial and central aspects of the caudate. Thus, anti-streptococcus IgM induces marked and subregion-specific increases in Fos expression in the caudate nucleus.

4.2. Nucleus accumbens

Fos-like immunoreactivity likewise varied as a two-way interaction between Antibody Treatment and Brain Subregion in the nucleus accumbens ($F(1,22) = 8.01$, $p < 0.01$; Fig. 3). Fos expression was significantly elevated in the shell and the core compartments of the nucleus accumbens in anti-streptococcus IgM-treated mice compared to their respective controls. Of further significance, the magnitude of IgM-induced increases in Fos in the shell significantly exceeded that evident in the core. Thus, anti-streptococcus IgM significantly stimulated activity in the nucleus accumbens in a subregion-specific manner.

4.3. Motor Cortex

In motor cortex, Fos-like immunoreactivity varied as an interaction between Antibody Treatment and Brain Subregion ($F(1,22) = 5.14$, $p < 0.05$; Fig. 4). Compared to their respective controls, Fos expression was significantly increased in primary (M1) and

secondary (M2) divisions. The number of Fos-positive cells in the M2 division exceeded that of the M1.

In summary, we show for the first time that anti-streptococcus IgM antibodies induce pronounced increases in Fos expression in CSTC circuits that are implicated in neuropsychiatric disorders and that are presumed to be targeted by antibodies associated with GABHS infections. Of further importance, there is a subregion specificity regarding the magnitude of these effects.

5. Anti-streptococcus IgM localizes within cortico-striatal regions

As illustrated in Fig. 5, anti-streptococcus IgM antibodies localized within the caudate (Fig. 5A), nucleus accumbens (Fig. 5B and C), and motor cortex (Fig. 5D and E). However, no labeling was found in insular cortex (Fig. 5F). Primary antibody omission controls were performed to control for nonspecific binding; no labeling was found in both saline or IgM treated brains (Fig. 5G). Indeed, there is a striking similarity in the distributions of IgM deposits and Fos-like immunoreactivity, possibly suggesting that IgM induces local effects in these brain regions. To be sure, future studies are required to shed light on this issue.

6. Fcα/μ Receptors are Expressed in the Striatum and Cortex and Co-Localize with Anti-Streptococcus IgM

We found that anti-streptococcus IgM accumulates in- and activates brain regions associated with CSTC motor circuits implicated in TS and other tic disorders. IgM antibodies bind to Fcα/μR on immune cells, and there is evidence that they are present in the subventricular zone of the lateral ventricle and the adjacent corpus callosum. However, there is no evidence that Fcα/μR's are expressed in CSTC motor circuits. Accordingly, we determined whether Fcα/μR's: (1) are expressed in the caudate, nucleus accumbens, and motor cortex, and (2) co-localize with anti-streptococcus IgM in these regions.

As shown in Figure 6, Fcα/μR's are widely distributed in the caudate, nucleus accumbens, and motor cortex (Fig. 6C, H, and M) in control mice. In anti-streptococcus IgM-treated animals, IgM co-localizes with Fcα/μR's in these regions (Fig. 6E, J, and O). To our knowledge, this is the first demonstration that Fcα/μR's are expressed in CSTC motor circuits, and that they co-localize with IgM in the brain. These findings support our view that anti-streptococcus IgM-induced alterations of cell activity reflect local actions of IgM and suggest that such effects involve Fcα/μR's. As shown in Fig. 6P–R, there was no evidence of labeling of Fcγ II/III R's, which bind IgG, in regions that demonstrated labeling of Fcα/μR's (Fig. 6C, D, H, I, M, and N). This observation provides further evidence of the specificity of the labeling of Fcα/μR's.

7. Discussion

In the present study we identify for the first time a role for anti-streptococcus IgM class antibodies in triggering repetitive stereotyped movements. There is an exquisite specificity to these effects as anti-streptococcus IgG and anti-KLH IgM induced a different pattern of behavioral changes. Anti-streptococcus IgM antibodies also accumulated and induced functional changes in CSTC motor circuits implicated in GABHS-related behavioral disturbances, including subregions of the caudate, nucleus accumbens, and motor cortex. Of additional importance, we discovered that Fcα/μR's, which bind IgM antibodies, are present in these brain regions and co-localize with anti-streptococcus IgM. Collectively, these findings support the use of anti-streptococcus monoclonal antibody administration in Balb/c

mice to model GABHS-related behavioral disturbances and to identify underlying neural mechanisms.

Anti-streptococcus IgM antibody-induced repetitive stereotyped movements included head bobbing, intense grooming, and sniffing. These stereotyped movements are analogous to either complex tics (e.g., head shaking scratching, and touching) or simple tics (e.g., sniffing) that are evident in patients suffering from disorders in which GABHS infections are implicated (Albin and Mink, 2006). In patients, such repetitive stereotyped movements are expressed as goal directed behaviors whose purpose is to relieve the urge or compulsion to tic. Thus, it will be of interest to determine the effects of anti-streptococcus IgM on goal directed and compulsive behaviors. Of further importance, given the connectivity and overlapping neuroanatomical substrates governing repetitive and habitual behaviors, future studies ought to determine whether anti-streptococcus IgM affects habit formation. Such analyses are additionally indicated given the co-morbid expression of repetitive stereotyped and obsessive-compulsive behaviors as well as other psychopathological outcomes in patients suffering from GABHS-related disorders. The present findings are also relevant to other neuropsychiatric disorders in which an increased expression of repetitive stereotyped movements and habitual behaviors are linked to elevated antibody titers. A notable example is autism spectrum disorder. Anti-nuclear antibody titers are elevated in mothers of children with autism spectrum disorder. It is thus of unique interest that exposing Rhesus monkeys prenatally to IgG class antibodies from such individuals results in increased whole-body stereotypies (Martin et al., 2008; see Ashwood and Van de Water, 2004). Of further importance, in mice, *in utero* exposure to IgG from mothers of children with autism spectrum disorder results in hyperactivity in an open field and anxiety-related behavior in the offspring (Singer et al., 2009).

In contrast with anti-streptococcus IgM, anti-streptococcus IgG did not appreciably affect head bobbing, intense grooming, or sniffing. Thus, the effects of IgM on these stereotypies are antibody class specific. The behavioral effects of anti-streptococcus IgM can be further contrasted with those induced by anti-streptococcus IgG. For example, while IgM reduced the expression of vertical stereotypic movements, IgG increased these movements. Moreover, IgG treatment induced significant increases in locomotion, and rearing whereas IgM had no effect. It is important to note that the present motor effects induced by anti-streptococcus mouse monoclonal IgG antibodies are consistent with those induced in mice immunized and boosted with a GABHS homogenate or purified IgG from streptococcus infected mice (Hoffman et al., 2004; Yaddanapudi et al., 2009). It is also noteworthy that in these studies, sera from GABHS-immunized mice were immunoreactive to brain regions implicated in GABHS infections (Hoffman et al., 2004; Yaddanapudi et al., 2009), and that increased motor activity was associated with IgG deposits in the brain. One difference between these studies is that we presently used monoclonal antibodies directed against anti-streptococcus IgG2a whereas Yaddanapudi and colleagues (Yaddanapudi et al., 2009) used GABHS mice in which IgG1 responses predominate. It should be pointed out that the profile of IgG subclasses in patients suffering from GABHS-related disorders remains to be elucidated. Nonetheless, the fact that identical changes in motor activity were induced across studies supports the use of subcutaneous injections of monoclonals against anti-streptococcus antibodies to model GABHS-related behavioral disturbances. Inasmuch as anti-streptococcus IgM and IgG antibodies induce different behavioral profiles, we conclude that these anti-streptococcus antibody classes play unique roles in provoking behavioral disturbances following GABHS infections. It seems relevant to note at this juncture that IgM, which is produced in high concentrations post-streptococcus infection, peaks 5–13 days post-infection in humans, while IgG antibodies appear around day 13. Thus, anti-streptococcus IgM may initially trigger symptoms post-infection.

In the present investigation, anti-streptococcus IgM-induced stereotypies were antigen-specific, and thus, not a general property of IgM class antibodies. Specifically, mice treated with single injections of anti-KLH IgM displayed decreases in novelty-induced rearing, head bobbing, and sniffing. It remains to be determined whether these antibody-induced behavioral disturbances reflect depressive-like behavior, and thus, whether antibodies acts as mediators of sickness behavior during an orchestrated immune response (see Dantzer et al., 2008; Fleshner et al., 2001; Anisman and Merali, 2003; Gaykema and Goehler, 2011; Zalcman et al., 1994).

In GABHS-related disorders, anti-neuronal antibodies are thought to alter activity in the basal ganglia and in anatomically related regions of cortex (Husby et al., 1976; Church et al., 2002; Kiessling et al., 1993; Kirvan et al., 2006). Previously, there was no in vivo evidence showing that anti-streptococcus IgM antibodies induce functional changes in such regions. However, in the present study, we found that anti-streptococcus IgM induced pronounced increases in Fos expression in the caudate, nucleus accumbens, and motor cortices. It is of interest to note that the central aspect of the caudate receives input from motor cortices, while the medial aspect receives input from the orbitofrontal and prefrontal cortices (Selemon and Goldman-Rakic, 1985). Both of these regions contribute to the expression of motor processes and goal directed aspects of motor function and learning (Ragsdale and Graybiel, 1981; Balleine and O'Doherty, 2010). Of further importance, c-Fos induction in the central caudate is associated with the expression of focused stereotypy (Chartoff et al., 2001). Inasmuch as anti-streptococcus IgM induced subregion-specific increases in Fos expression in the caudate nucleus, it will be of interest to determine the relative contributions of these subregions to the present behavioral disturbances. Moreover, the linkage between repetitive stereotypic movements and anatomical loci has received support from the clinical literature. Specifically, imaging studies have shown activation of regions including neostriatum, motor and premotor cortices, and prefrontal cortex in patients suffering from tic disorders (e.g., Stern et al., 2000; Bohlhalter et al., 2006; see Albin and Mink, 2006).

Anti-streptococcus IgM treatment induced pronounced increases in Fos expression in the nucleus accumbens, which serves as an interface between motor activity and motivation (Mogenson et al., 1980). It receives inputs from mesocorticolimbic structures, including the ventral tegmental area, amygdala, ventral hippocampus and prefrontal cortices. The accumbens projects to additional components of CSTC circuits that are implicated in tic and movement disorders, including the ventral pallidum and substantia nigra (Bohlhalter et al., 2006). The shell region of the accumbens, which was markedly activated by anti-streptococcus IgM, plays a pivotal role in underlying predetermined motor responses to unconditioned stimuli. Of further importance, the core region of the accumbens, which was also targeted by IgM, translates the context and/or motivational value of a stimulus into motor responses (see Meredith et al., 2008). Because anti-streptococcus IgM induced subregion-specific alterations in Fos expression in the accumbens, it will be important to identify the roles of the shell and core compartments in underlying the observed behavioral variations. Inasmuch as motivated behavior with negative or positive valence appears to be organized along rostrocaudal gradients in the nucleus accumbens (Reynolds and Berridge, 2008), future studies should also identify the effects of anti-streptococcus IgM on microcircuits within these gradients.

It is also noteworthy that anti-streptococcus IgM increased Fos expression in the M1 and M2 divisions of motor cortex. Particularly robust activation of the M2 division of was observed. Given its role in fine motor control and its anatomical connections with regions of the caudate shown to be activated in the present study, it is suggested that this cortico-neostriatal circuit also plays a role in underlying motor stereotypies such as head bobbing, sniffing, and

intense grooming. Other regions would of course also be expected to be involved. For example, in association with the circuits described above, grooming is linked with alterations in nigrostriatal activity. It is thus of unique relevance that compulsive grooming is evident in autoimmune mice, and that such effects are related to aberrations in nigrostriatal activity (Chun et al., 2008).

Inspection of the data revealed that the stereotypy-inducing effects of anti-streptococcus IgM appeared within 40-min of injection. This time frame is similar to that required for peripherally administered IgM class antibodies to enter and accumulate in the brain (Banks et al., 2007). The mechanisms by which anti-streptococcus IgM enters the brain remain to be determined. To be sure, an intact BBB is required to preserve CNS homeostasis. Antibodies have been shown by Broadwell and Sofroniew (1993) and IgM in particular by Banks (2007) to cross the intact BBB. Stress, neuroinflammation, and other factors which are known to disrupt the BBB could possibly further contribute to the ability of antibodies to enter the brain. The mechanisms by which anti-streptococcus IgM enters the brain remain to be determined. To be sure, an intact blood-brain barrier (BBB) is required to preserve CNS homeostasis. Typically, peripheral antibodies do not readily cross the BBB. However, entry may occur through weakened portions of the BBB or in the presence of permissive factors such as LPS, epinephrine, or stress. In the former regard, Broadwell and Sofroniew (1993) showed that IgG and IgM may gain access into the CNS via circumventricular organs. Thus, it is possible that anti-streptococcus IgM entered the brain through such pathways. Regarding permissive factors, Diamond and colleagues showed in Balb/c mice that anti-DNA antibodies that bind a pentapeptide consensus sequence in the NMDA receptor gain access to the brain when the BBB is breached by lipopolysaccharide (LPS) (Kowal et al., 2004) or by systemic injections of epinephrine (Huerta et al., 2006). Of further importance, various investigators have shown that stressors may transiently increase permeability of the BBB (e.g., Ben-Nathan et al., 1991; Dvorská et al., 1992; Sharma et al., 1991). Inasmuch as mice were presently tested under stressful conditions (i.e., novelty stress) coupled with the fact that Balb/c mice are stress vulnerable, it is possible that stress presently acted as a permissive factor.

We presently found that anti-streptococcus IgM localizes in the caudate, nucleus accumbens and motor cortices. Of additional importance, we discovered that Fc α / μ R's, which bind IgM antibodies, are present in CSTC motor circuits, including the caudate, nucleus accumbens, and motor cortex. A striking finding is that anti-streptococcus IgM co-localizes with Fc α / μ R's in brain regions stimulated by IgM. Based on these findings, we propose a novel hypothesis regarding mechanisms underlying GABHS-related behavioral disturbances, namely that anti-streptococcus IgM binds to Fc α / μ R's in the striatum and cortex, induces functional changes in cell activity, which in turn triggers repetitive stereotyped movements. It should be noted that in the caudate, nucleus accumbens, and motor cortex there was no evidence of labeling of Fc γ II/III R's, which bind IgG, thus demonstrating the specificity of Fc α / μ R labeling. It is uncertain whether Fc γ II/III R's are expressed in other motor regions of the brain, as reported for Fc γ IIbR in the cerebellum (Nakamura et al., 2007). It also remains to be determined whether Fc γ R1, which bind IgG with high affinity, or other classes of Fc γ R's are expressed in the cortex and striatum.

In immune cells, the binding of an antibody to its FcR may activate tyrosine kinases, ERK1/2 MAP kinase pathway and serine/threonine kinases (see Okun et al., 2010). The relevance here is that possible IgM-Fc α / μ R interactions in the brain may stimulate such pathways. As such, FcR's may represent novel therapeutic targets in neurological and psychiatric disorders in which antibody molecules act as etiological agents. Moreover, FcR activation in immune cells may stimulate or inhibit cytokine production. Thus, GABHS infection-related anti-streptococcus IgM antibodies may influence the peripheral and/or

central release of cytokines that potentiate cell activity in CSTC motor regions or the inhibition of cytokines that suppress cell activity in such regions. This view is supported by evidence that inflammatory cytokines induce (Petitto et al., 1997; Zalcman et al., 1998; Zalcman, 2002) or suppress motor activity (Nakajima et al., 2004), and the finding that cytokines are increased in patients suffering from tic disorders (Leckman et al., 2005; Morer et al., 2010).

In light of its modulatory effects on the caudate, nucleus accumbens, and motor cortex, it is reasonable to suggest that new insights into our understanding of TS, OCD, and other disorders associated with abnormal basal ganglia function (such as ASD) may be gained from further systematic analyses of the relationships between antibodies and their neuroanatomical substrates, and of the role of Fc receptors in mediating antibody-induced variations in brain activity.

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Fig. 1.

Anti-streptococcus IgM antibody-induced repetitive stereotyped movements. Mean ($\pm$ SEM) activity scores for (A) Head bobbing, (B) Intense grooming, (C) Sniffing, (D) Total stereotypy score, and (E) Stereotypy score at various time intervals (min) following single injections of anti-streptococcus IgM antibodies (0, 6.25, 12.5 μ g/mouse, sc; $n = 7-9$ /group).

Fig. 2. Photomicrographs of brain sections showing Fos-like immunoreactive cells in the caudate-putamen of mice receiving single injections of (A) saline or (B) anti-streptococcus IgM. Inserts in (A) and (B) reflects regions magnified by 20 $\times$ in (C) and (D) respectively. (E) Histogram show the number (mean $\pm$ SEM) of Fos-positive cells counted within the indicated subterritories of caudate-putamen after administration of saline or anti-streptococcus IgM ($n = 6-7$ /group). Insert reflects regions sampled for counting Fos-positive cells in the dorsomedial (DM), dorsolateral (DL), central (CT), ventrolateral (VL) and ventromedial (VM) aspects of the caudate-putamen.

Fig. 3. Photomicrographs of brain sections showing Fos-like immunoreactive cells in the nucleus accumbens of mice receiving single injections of (A) saline or (B) anti-streptococcus IgM. Inserts in (A) and (B) reflects regions magnified by 20× in (C) and (D), respectively. (E) Histogram show the number (mean ± SEM) indicate Fos-positive cells counted within the indicated compartments of the nucleus accumbens after administration of saline or anti-streptococcus IgM ($n = 6-7$ /group). CPu, caudate-putamen; aca, anterior commissure.

Fig. 4. Photomicrographs of brain sections demonstrating Fos-like immunoreactive cells in motor cortex of mice receiving single injections of (A) saline or (B) anti-streptococcus IgM. (C) Histogram shows the number (mean $\pm$ SEM) of Fos-positive cells counted within the indicated compartments of the motor cortex after administration of saline or anti-streptococcus IgM ($n = 6-7$ /group). Abbreviations: M1, primary motor cortex; M2, secondary motor cortex.

Fig. 5. Photomicrographs of anti-streptococcus IgM deposits in (A) caudate–putamen, (B) shell of the nucleus accumbens, (C) core of the nucleus accumbens, (D) M1 division of motor cortex, (E) M2 division of motor cortex and (F) insular cortex of mice receiving single injections of saline or anti-streptococcus IgM ($n = 6-7/\text{group}$). (G) omission control in caudate of mice receiving single injections of saline or anti-streptococcus IgM.

Fig. 6.

Photomicrographs of anti-streptococcus IgM and Fc Receptors (FcR) in caudate-putamen (CPu), nucleus accumbens (Acb) and M2 division of motor cortex. (A) Absence of anti-streptococcus IgM labeling in CPu of saline-treated animals; (B) presence of anti-streptococcus IgM labeling in CPu of anti-streptococcus IgM treated animals; (C and D) Fcα/μR labeling in CPu of both saline-treated and anti-streptococcus IgM treated animals; (E) Co-localization of anti-streptococcus IgM and Fcα/μR in CPu of anti-streptococcus IgM treated mice. Arrows: illustration of both IgM and Fcα/μR labeling in CPu; arrowhead: example of only single label of IgM in CPu; (F) Absence of anti-streptococcus IgM labeling in nucleus accumbens (Acb) of saline-treated animals; (G) presence of anti-streptococcus IgM labeling in Acb of anti-streptococcus IgM treated animals; (H and I) Fcα/μR labeling in Acb of both saline-treated and anti-streptococcus IgM treated animals; (J) Co-localization of anti-streptococcus IgM and Fcα/μR in Acb of anti-streptococcus IgM treated mice. Arrows: illustration of both IgM and Fcα/μR labeling in Acb; arrowhead: example of only single label of IgM in Acb; (K) Absence of anti-streptococcus IgM labeling in M2 (secondary motor cortex) of saline-treated animals; (L) presence of anti-streptococcus IgM labeling in M2 of anti-streptococcus IgM treated animals; (M and N) Fcα/μR labeling in M2 of both saline-treated and anti-streptococcus IgM treated animals; (O) Co-localization of anti-streptococcus IgM and Fcα/μR in M2 of anti-streptococcus IgM treated mice. Arrows: illustration of both IgM and Fcα/μR labeling in M2; arrowhead: example of only single label of IgM in M2; (P, Q and R) Absence of Fcγ II/IIIIR labeling in caudate, nucleus accumbens and motor cortex of saline-treated animals.

Table 1

Effects of anti-streptococcus IgM antibodies on behaviors related to those illustrated in Fig. 1.

Parameter	Saline	6.25 µg anti-strep IgM	12.5 µg anti-strep IgM
Turns	41.33 ± 4.56	38.88 ± 6.29	26.5 ± 13.40
Horizontal stereotypic movements	472.17 ± 23.86	410.75 ± 20.11	373.67 ± 49.53
Vertical stereotypic movements	100.00 ± 11.02	80.00 ± 29.45	102.00 ± 36.65
Rearing	142.83 ± 12.29	164.89 ± 22.78	157.71 ± 24.66
Ambulatory distance (cm)	9172.08 ± 1323.65	7309.33 ± 849.53	7659.90 ± 1547.79

Table 2

Anti-streptococcus IgG antibody-induced behavioral changes.

Parameter	Saline	6.25 µg anti-strep IgG	12.5 µg anti-strep IgM
Head bobbing (s)	20.14 ± 6.57	10.33 ± 3.70	14.33 ± 6.29
Intense Grooming (s)	65.33 ± 7.89	94.00 ± 14.20	80.00 ± 13.88
Sniffing (s)	155.00 ± 17.84	194.17 ± 23.60	230.85 ± 28.21
Vertical stereotypic movements	37.00 ± 11.19	43.75 ± 11.91	243.5 ± 33.18 ^{**}
Rearing	81.29 ± 18.23	102.33 ± 14.22	20.14 ± 27.38 [*]
Ambulatory distance (cm)	6330 ± 951.37	5896.10 ± 553.50	8996.55 ± 1821.8 [*]

^{*}
 $p < 0.05$.

^{**}
 $p < 0.001$.

Table 3

Anti-KLH IgM antibody-induced behavioral changes.

Parameter	Saline	6.25 µg anti-KLH IgM	12.5 µg anti-strep IgM
Head bobbing (s)	22.57 ± 5.61	11.29 ± 3.08	7.42 ± 3.12 *
Intense Grooming	132.33 ± 26.02	108.67 ± 23.82	131.5 ± 13.54
Sniffing (s)	320.0 ± 34.89	145.6 ± 27.39 **	146.5 ± 35.37 **
Vertical stereotypic movements	35.17 ± 4.47	27.71 ± 14.13	26.50 ± 6.76
Rearing	99.00 ± 14.61	32.00 ± 14.57 *	58.33 ± 15.75
Ambulatory distance (cm)	7198.91 ± 786.08	5554.33 ± 491.35	6625.57 ± 620.94

*
 $p < 0.05$.**
 $p < 0.01$.